Limit-cycle oscillations and chaos in reaction networks subject to conservation of mass

(nonlinear dynamics/free-energy transduction/linkage)

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Contributed by Jeffries Wyman, August 1, 1988

ABSTRACT A cyclic network of autocatalytic reactions involving an unbuffered cofactor and a number of components subject to conservation of mass displays a surprising richness of dynamical behaviors. Limit-cycle oscillations are possible over a wide range of parameter values. Additionally, a cascade of period-doubling bifurcations leading to chaos can coexist with a multiplicity of stable steady states. These results draw attention to the role of unbuffering as a feedback in biochemical systems.

Periodic patterns of temporal self-organization may appear in several reactions and biochemical regulatory processes when the system under consideration obeys certain general properties (1): (i) it is open to mass or energy transfer; (ii) it is far from equilibrium whereby the individual reaction steps are mostly irreversible; and (iii) the chemical reaction mechanism is nonlinear. Consistent with these criteria we have drawn attention (2) to the existence of stable, conservative chemical oscillations in cyclic, autocatalytic reaction networks of n components obeying the general scheme

where X_i denotes the components, K_i is a kinetic constant, $i = 1 \dots n$, and $X_{i+n} = X_i$. The network is driven far from equilibrium by a constant energy source included in the kinetic constants. The change in the concentration x_i of X_i is

$$dx_i/dt = x_i(K_{i-1}x_{i-1} - K_i x_{i+1}), \quad [2]$$

which indicates that X_i is formed by interaction with the preceding component and destroyed by interaction with the successive one. The sum of the values of x is independent of time, so that the system is closed with respect to the components X , and open to external free-energy sources that drive the unidirectional circulation of the network. The topology of these networks and their properties dependent upon n have a close connection to neural networks (3) and to competing populations in closed systems (4). Conservation of the components is a feature of particular interest when dealing with biological macromolecules. In this case the components can be considered as different conformational or ligation states of a biological macromolecule circulating in a reaction network, as in the case of the turning-wheel model (5) or the linear networks extensively analyzed by Hill (6). Conservation of mass corresponds to the fact that the macromolecule is present in fixed amounts in a fixed volume, and Eq. 2 underlies the autocatalytic nature of the interconversions among the states of the macromolecule.

Although the networks described by Eq. 1 allow only for

conservative oscillations, they can be used as skeletons in other reaction mechanisms to generate complex dynamical behavior. In this paper we demonstrate with a particular model that under suitable conditions the conservative nature of the oscillations is lost and these networks can display a surprising richness of dynamical behaviors, from limit-cycle oscillations to a cascade of period-doubling bifurcations leading to chaos. A peculiar feature of the model is the possibility of either periodic oscillations or chaos coexisting with a multiplicity of stable steady states.

THE MODEL

The particular model considered here is a four-component autocatalytic network, such as Eq. 1, driven from the outside by a constant energy source. The basic assumption is that, in addition to the components X_1 , X_2 , X_3 , and X_4 , the reaction network now includes a cofactor that is not degraded and is present in a fixed amount. For a chemical reaction, an example of such a cofactor is provided by a proton taken up at a given step and released in another one. The reaction scheme is

where Y denotes the cofactor that binds to X_1 to give X_2 and is released from X_2 to give X_3 . The differential equations for the above system are

$$dx_1/dt = x_1(K_4x_4 - K_1yx_2) \quad [4a]$$

$$dx_2/dt = x_2(K_1yx_1 - K_2x_3) \quad [4b]$$

$$dx_3/dt = x_3(K_2x_2 - K_3x_4) \quad [4c]$$

$$dx_4/dt = x_4(K_3x_3 - K_4x_1), \quad [4d]$$

where x_i is the concentration of the various components and y is the cofactor activity. The sum of Eq. 4 a-d is always zero, independent of time, because the system is closed to the various components of X .

If the cofactor is present in large excess with respect to the components, then it is buffered and its activity does not change during circulation of the reaction network. In this case the effect of the cofactor can be included in the kinetic constants, and the system shows conservative oscillations if $K_1K_3y = K_2K_4$, or decays toward a stable steady state otherwise (2). If the cofactor is not buffered then its activity may

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drastically change and affect the rate of the reaction in which it is involved. The activity y is written as $y = m - x_2$, where m is the total concentration of cofactor and x_2 gives the amount of cofactor bound to the component X_2 . As a consequence, nonlinear terms of the third order appear in Eq. 4. For the sake of simplicity, but without loss of generality, we normalize the total concentration of the components to 1, so that the steady-state values for the components are given by

$$\bar{x}_1 = \frac{1 - (m - K_2K_4/K_1K_3)(1 + K_2/K_3)}{(1 + K_4/K_3)} \quad [5a]$$

$$\bar{x}_2 = m - K_2K_4/K_1K_3 \quad [5b]$$

$$\bar{x}_3 = \frac{K_4}{K_3} \bar{x}_1 \quad [5c]$$

$$\bar{x}_4 = \frac{K_2}{K_3} \bar{x}_2. \quad [5d]$$

Unbuffering the system introduces constraints among the autocatalytic constants that define a unique steady state. The positivity of the values of $\bar{x}$ leads to the requirement

$$\left(\frac{K_2K_4}{K_1K_3}\right) + \left(\frac{K_3}{K_2 + K_3}\right) > m > \frac{K_2K_4}{K_1K_3}, \quad [6]$$

which indicates that a steady state is only possible when the total concentration of cofactor lies within this range defined by the kinetic constants, with the total mass of the macro-molecule normalized to 1.

The stability of solutions to Eq. 4 is obtained by linearizing about the values of $\bar{x}$ and investigating the eigenvalues ω of normal mode analysis (7). Three eigenvalues are given by the roots of the secular equation

$$\begin{aligned} \omega^3 + K_1\bar{x}_1\bar{x}_2\omega^2 + (K_2K_4/K_3)\bar{x}_1\bar{x}_2 \\ \times (2K_2K_4/K_3 + K_2 + K_3 + K_4 - K_1m)\omega \\ + (K_1K_2K_4/K_3)(K_3 + K_4)\bar{x}_1^2\bar{x}_2^2 = 0. \end{aligned} \quad [7]$$

The fourth eigenvalue is precisely zero due to conservation of mass. One eigenvalue is always real and negative and the remaining two have a common real part that becomes positive when

$$K_1m > \frac{K_2}{K_3}(K_3 + 2K_4), \quad [8]$$

which follows from the Hurwitz criterion (7). The equality sign yields a Hopf bifurcation. Combination of Eqs. 6 and 8 gives a relation among the kinetic constants for the necessary, although not sufficient, condition for existence of a limit cycle as

$$K_3^2(K_1 - K_2) > K_2(K_2K_4 + K_3K_4 + K_2K_3), \quad [9]$$

which is independent of m . This relation implies that the steady state can become unstable when the rate of cofactor binding K_1 is greater than the rate of release K_2 .

RESULTS

The key dynamical features of the model are analyzed by studying the dynamics of the system as a function of K_1 , K_2 , and m , while K_3 and K_4 are held constant. We first study the effect of changing K_2 and m for assigned values of the other kinetic constants. For the sake of simplicity we assume that $K_1 = K_3 = K_4 = 1$. Fig. 1 shows the effect of changing K_2

Fig. 1. Dynamical properties of the system as a function of the kinetic constant K_2 and the concentration of cofactor m . The other kinetic constants are equal to 1. Region A is the region where oscillations decay exponentially, and region B is the region of limit-cycle oscillations. The boundary across these regions is the Hopf bifurcation. In regions C no steady state is possible. The boundaries between the regions C and those where a steady state exists (A and B) are characterized by a power-law decay of the oscillations. At the upper boundary the concentrations of components X_1 and X_3 are zero. The same holds for the components X_2 and X_4 at the lower boundary.

and m on the dynamical properties of the system. The Hopf bifurcation separates the region A of exponential damping of the oscillations from the region B where limit cycles appear. The region characterized by the presence of a limit cycle covers a wide range of parameter values. The boundaries separating the regions A and B from the regions C set the domain wherein the system has a steady state. At the upper boundary $\bar{x}_1 = \bar{x}_3 = 0$ and at the lower boundary $\bar{x}_2 = \bar{x}_4 = 0$. Both boundaries correspond to the unusual dynamical condition that all eigenvalues of Eq. 8 are precisely zero. An interesting consequence is that on these boundaries the steady state is approached according to an inverse power law similar to that found for the four-component hypercycle (8). This dynamical behavior is an example of quasistability in that the system mimics a limit cycle over long time periods and then ultimately, or adiabatically, decays toward the steady state. However, as distinct from the hypercycle, the present power-law decay is accompanied by oscillations that increase in period.

Second, we study the effect of changing K_1 and m on the dynamical behavior of the system keeping all the other kinetic constants equal to 1. The results are shown in Fig. 2. Again, the regions where the system has a steady state (A, B, and B') are limited by two boundaries, and the Hopf bifurcation separates the region A of exponential damping of oscillations from region B within which limit-cycle oscillations are present. In region B there is another cut (dotted line) that gives rise to an interesting dynamical phenomenon. Eq. 5 is the nontrivial steady-state solutions of Eq. 4. However, in addition to these solutions, there is an infinite number of trivial solutions. For example, for $x_1 = x_3 = 0$ an infinite number of combinations of $x_2 + x_4 = 1$ satisfies Eq. 4. The same holds for any $x_1 + x_3 = 1$ when $x_2 = x_4 = 0$. Separate stability analysis of these trivial solutions shows that they are all unstable for any values of the kinetic constants and m , except for a region of the parameter space where the particular solution $x_1 = x_3 = 0$, $x_2 = \alpha$, and $x_4 = 1 - \alpha$, with $0 < \alpha < 1$.

FIG. 2. Dynamical properties of the system as a function of the kinetic constant K_1 and the concentration of cofactor m . The other kinetic constants are equal to 1. Regions C are the regions where no steady state exists. Region A is the region where oscillations decay exponentially, and region B is the region of limit-cycle oscillations. The boundary across these regions is the Hopf bifurcation. The dotted line ($\Delta = 0$) separates the regions B ($\Delta < 0$) and B' ($\Delta > 0$) and gives the split bifurcation where a second attractor appears in the system. In the region B' a cascade of period-doubling bifurcations progressively culminates with chaos, and the basin of initial conditions leading to this dynamical behavior coexists with another basin that leads to fuzzy steady states.

m , becomes stable. This region is defined by the condition

$$(K_4 + K_1 m)^2 - 4K_1 K_4 = \Delta \geq 0, \quad [10]$$

which depends only on K_1 , K_4 , and m . The surface bounding the region is given by $\Delta = 0$. This surface projected in the K_1, m parameter space gives the cut shown in Fig. 2 (dotted line) in the domain where limit-cycle oscillations are possible. At this boundary a split bifurcation (9) doubling the number of stable steady states is observed. One steady-state solution is given by Eq. 6 and the other one is given by the coordinates

$$\bar{x}_1 = 0 \quad [11a]$$

$$\bar{x}_2 = \sqrt{K_4/K_1} \quad [11b]$$

$$\bar{x}_3 = 0 \quad [11c]$$

$$\bar{x}_4 = 1 - \sqrt{K_4/K_1}. \quad [11d]$$

The appearance of a second steady state coincides with the onset of a period-doubling bifurcation in the system. When $\Delta > 0$, the cut in Fig. 2 is crossed and the region B' is entered. Here an infinite number of attracting points, or fuzzy steady states, appear in the interval

$$\bar{x}_1 = 0 \quad [12a]$$

$$\frac{K_4 + K_1 m - \sqrt{\Delta}}{2K_1} < \bar{x}_2 < \frac{K_4 + K_1 m + \sqrt{\Delta}}{2K_1} \quad [12b]$$

$$\bar{x}_3 = 0 \quad [12c]$$

$$\bar{x}_4 = 1 - \bar{x}_2, \quad [12d]$$

which compete with the attractor defined by Eq. 5. Fuzzy

refers to a continuous locus of stable steady-state points, each of which depends upon the particular choice of initial conditions. Fuzzy steady states can be achieved whenever a coordinate passes through a zero. This possibility is inherent to autocatalytic networks that comply with Volterra models (4). The presence of fuzzy steady states triggers a cascade of period-doubling bifurcations, as shown in Fig. 3 *a-d*. The cascade culminates with chaos (see Fig. 3*e*). This parallels the scenario observed in one-dimensional unimodal maps (9). The bifurcations are probably due to the destabilization of the oscillatory motion around the attractor defined by Eq. 5 induced by the new attractors, Eq. 12. The locus of new attractors increases with the value of m and eventually spans the whole range of possible values of the variables x_2 and x_4 . Thus, in region B' for a given set of initial conditions the system will show a cascade of period-doubling bifurcations leading to chaos, and for a different set of initial conditions the trajectory in the phase space will be attracted to the fuzzy steady states, Eq. 12. As a consequence periodic motion (period doubling) or chaos can coexist with a locus of stable steady states, unlike the case of bistability (9, 10) or birhythmicity (11) where periodic motion or chaos coexists with another periodic motion. The dynamics displayed by the model discussed here is thus characterized by the existence of two basins of initial conditions, one leading to period-doubling bifurcations and chaos, and the other leading to the region of fuzzy steady states. The structures of these basins are difficult to predict. In two-parameter maps that allow for bistability the basins consist of infinitely many intervals of fractal structure (10). In one-parameter maps, they are of simple structure in the period-doubling regime and of fractal structure in the aperiodic regime (12). At the boundaries of the basins, the system can undergo abrupt changes after small changes in the parameter values. The periodic or chaotic motion can suddenly be damped and, vice versa, the trajectory trapped in the locus of sinks can suddenly wind up in a periodic or chaotic regime.

DISCUSSION

Autocatalytic reaction networks are of particular interest in the description of coupling processes, such as those involved in self-organizing systems (3). In general terms the autocatalytic networks, Eq. 1, provide a class of models for self-organization based on imperfect catalysis, or competition among the components of the system (2). Preliminary investigation shows that the dynamical behavior of the prototypic model discussed here is common to the entire class of autocatalytic networks, Eq. 1, in the presence of unbuffered cofactors. There is a strong indication that these networks may display a progressively more complex dynamical behavior as the number of components increases.

The topology of the networks plays a fundamental role in collapsing or expanding regions of particular time behaviors. For example, the power-law decay observed in the prototypic model, Eq. 3, only in particular transition cuts becomes the dominant dynamical feature of the system when the reaction scheme is modified to allow the cofactor to reenter the network. In this case quasistable oscillations are observed over a wide range of parameter values. Different effects arise by changing the position where the cofactor reenters the network or, more interestingly, by increasing the number of cofactors in the system. The abrupt changes observed at the boundaries of the basins of the attractors after a split bifurcation draw attention to possible biological implications of the coexistence of multiple periodicity and chaos with fuzzy steady states. This phenomenon provides a plausible mechanism for the onset of sudden chaotic behavior in an otherwise stable system. Perhaps it may be used to complement the phenomenon of bistability (9, 10) in the analysis of

FIG. 3. Cascade of period-doubling bifurcations triggered by a change in the total concentration of cofactor m in the x_4x_3 phase space. The values of the kinetic constants are: $K_1 = 10 \text{ sec}^{-1}$ and $K_2 = K_3 = K_4 = 1 \text{ sec}^{-1}$. The initial conditions are: $x_1 = 0.97$ and $x_2 = x_3 = x_4 = 0.01$, in all cases. Transients are not shown. (a) $m = 0.5$, period-1. (b) $m = 0.56$, period-2. (c) $m = 0.57$, period-4. (d) $m = 0.572$, period-8. (e) $m = 0.575$, chaos. (e) Trajectory from $t = 0$ to $t = 1400 \text{ sec}$. The coexistence of two basins of attraction can readily be illustrated in c by using the initial conditions: $x_1 = x_2 = x_4 = 0.1$ and $x_3 = 0.7$ or $x_1 = x_2 = x_4 = 0.09$ and $x_3 = 0.73$, which drive the trajectory to two values of x_4 in the locus of fuzzy steady states (0.617 and 0.595, respectively).

the complex behavior of neural networks. Fuzzy steady states can only be prescribed in terms of a locus of points specified by an inequality such as Eq. 12. Their precise values, depending as they do on initial conditions, must be established by computation. Therefore, they are *a priori* indeterminate.

These results can also be discussed in connection with bio-

chemical systems, where the various components of the networks can be viewed as different allosteric states of a polyfunctional macromolecule (2). Autocatalysis necessarily demands direct collision of the macromolecular states, as observed in biological membranes where proteins are closely packed (13). When a cofactor is involved in the autocatalytic reaction network and its activity changes drastically during

circulation of the macromolecule, then the conservative nature of the network is lost and the system shows a complex dynamical behavior. The presence of an unbuffered cofactor may lead to limit-cycle oscillations over a wide range of parameter values, which allows the allosteric states of the macromolecule to function as a robust biochemical clock. Under particular conditions this allosteric clock could undergo a cascade of period-doubling bifurcations culminating with chaos. Cofactors are present in many biochemical control processes and in highly compartmentalized systems, such as biological membranes, they cannot be in contact with an infinite reservoir and are likely to be unbuffered. More generally unbuffering can occur whenever the rate of exchange of a substance with its reservoir is slow compared to the reaction rates in which the substance is involved.

Many of the patterns of temporal self-organization present in the model discussed here have been observed in chemical reactions involving biological macromolecules, such as those of the glycolytic chain (14, 15) or the peroxidase reaction (16). These patterns have been modeled by assuming that the macromolecular states are at steady state and that the periodic changes in substrate and product are due to feedbacks of these molecules mediated by the macromolecule (17–19). The pseudo-steady-state assumption of the macromolecular states is typically invoked in the study of biochemical systems, since it greatly simplifies the analysis, but it necessarily demands all the components involved in the reactions to be present in excess amount with respect to the macromolecule (20). The reaction scheme 3 draws attention to the fact that a complex dynamical behavior may arise in a biochemical system when unbuffered components are present.

All this raises a question as to the possible role of unbuffering in biochemical systems. Unbuffering a component introduces higher-order nonlinearity in the differential equations describing the time behavior of the system, which may give rise to a variety of dynamical behaviors. Interestingly, unbuffering has drastic effects also in systems at equilibrium, as expressed by the principle of the binding capacity (21), which provides both the direction of the effects and their magnitude, and the concept of pseudolinkage (22). This

suggests that biochemical systems can use unbuffering as a mechanism of regulation in equilibrium and nonequilibrium processes. This mechanism is model independent as it is intrinsic to the law of mass action.

This work was supported by National Science Foundation Grant PCM772062 (J.W.). E.D.C. gratefully acknowledges financial support from National Institutes of Health Grant HL22325 during his stay at the University of Colorado (Boulder).

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