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Allosteric regulation, cooperativity, and biochemical oscillations

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Allosteric regulation is associated with a number of periodic phenomena in biochemical systems. The cooperative nature of such regulatory interactions provides a source of nonlinearity that favors oscillatory behavior. We assess the role of cooperativity in the onset of biochemical oscillations by analyzing two specific examples. First, we consider a model for a product-activated allosteric enzyme which has previously been proposed to account for glycolytic oscillations. While enzyme cooperativity plays an important role in the occurrence of oscillations, we show that these may nevertheless occur in the absence of cooperativity when the reaction product is removed in a Michaelian rather than linear manner. The second model considered was recently proposed to account for signal-induced oscillations of intracellular calcium. This phenomenon originates from a nonlinear process of calcium-induced calcium release. Here also, the cooperative nature of that positive feedback favors the occurrence of oscillations but is not absolutely required for periodic behavior. Besides underlining the importance of cooperativity, the results highlight the role of *diffuse nonlinearities* distributed over several steps within a regulated system: even in the absence of cooperativity, such mild nonlinearities (e.g., of the Michaelian type) may combine to raise the overall degree of nonlinearity up to the level required for oscillations.

1. Introduction

Few concepts have proved so fertile in cellular regulation as that of allostery (refs 1–5; see ref. 6 for a review of its development). Besides their ubiquitous role which is to allow for highly sensitive responses to changes in the concentration of regulatory ligands, allosteric interactions are also involved in the origin of rhythmic phenomena at the cellular level. Indeed, because of their cooperative nature, they often provide the nonlinear kinetics that is a necessary prerequisite for the occurrence of oscillations. The purpose of this paper is to examine the role played by the cooperativity of allosteric interactions in the occurrence of biochemical oscillations.

From a thermodynamic point of view, sustained oscillations take place in open systems, once a nonequilibrium steady state becomes unstable as a result of appropriate nonlinearity of the kinetic equations [7]. Besides feedback processes, cooperativity provides a most important source of nonlinearity in biochemical kinetics. Since both sources are linked in allosteric regulation, it is not surprising that the latter has repeatedly been implicated in the mechanism of biochemical oscillations. The concept of allosteric interactions, as formalized in the classical model of Monod, Wyman and Changeux (MWC model) [2], provides a natural framework for cooperativity at the molecular level. This model is based on the concerted transition of the subunits of a protein between two distinct conformational states. In the alternative model proposed by Koshland et al.

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[4,5], such transitions are sequential rather than concerted. In either case, cooperative interactions between the subunits affect their propensity to undergo conformational transitions. Such cooperativity is responsible for the sigmoidal form of saturation or velocity curves; the stronger the cooperativity, the sharper the threshold that characterizes these curves.

What is the role of cooperativity in the occurrence of oscillations? Is it an absolute requirement, and if so what is its function in the mechanism of periodic behavior? Rather than addressing this question from the outset in a too general manner, we shall focus on two specific examples of biochemical periodicity pertaining to an oscillatory enzyme reaction and to signal-induced, repetitive calcium transients within the cell. Thus, we shall not consider the origin of oscillations in electrically excitable systems, as encountered in neurons or muscle cells. In these cases also, kinetic equations are highly nonlinear [8]; such nonlinearity may result in part from the allosteric nature of ion channels formed by multiple protein subunits [9].

The first example considered here relates to glycolytic oscillations in yeast and muscle; this phenomenon is the prototype of oscillatory behavior resulting from the regulation of an enzyme, phosphofructokinase, whose allosteric behavior has recently been unraveled at the molecular level [10]. A simple model for a product-activated allosteric enzyme accounts for most experimental observations on glycolytic oscillations. After recalling the prominent features of that model, we first summarize results previously obtained as to the role of cooperativity in the origin of periodic behavior. By considering a modification of the basic model, we then show how to bypass the requirement for cooperativity in order to generate oscillations in that system. The second periodic phenomenon to be addressed is that of intracellular calcium oscillations which occur in a variety of cells in response to neurotransmitter or hormonal stimuli. There also, the analysis of a model permits assessment of the role of the cooperativity of allosteric regulation in the origin and maintenance of oscillatory behavior. Taken together, the results confirm that cooperativity favors periodic behavior but highlight the potential role of *diffuse nonlinearities* in allowing for oscillations in the absence of cooperative interactions.

2. The product-activated allosteric enzyme: a prototype of biochemical oscillations

2.1. Allosteric model for glycolytic oscillations

Glycolytic oscillations are the best-known example of periodic behavior in biochemistry [11–13]. They occur in yeast and muscle cells as well as in yeast extracts when the continuous injection of a substrate such as glucose or fructose falls between two critical values [14]. All glycolytic intermediates then evolve in a rhythmic manner, with a period of the order of minutes. Soon after the discovery of this phenomenon some 25 years ago, it became clear that phosphofructokinase is the primary source of oscillatory behavior in glycolysis. The fact that the enzyme is activated by a reaction product, ADP or AMP – which is related to ADP through the adenylate kinase reaction – was identified as being responsible for the instability that leads to sustained oscillations [15]. Models based on the activation of an enzyme by a reaction product were proposed to account for oscillatory behavior [16,17].

The allosteric nature of phosphofructokinase was later considered explicitly in a model [18,19] based on the concerted transition theory developed by Monod, Wyman and Changeux [2]. The model for the product-activated allosteric enzyme is schematized in fig. 1. For simplicity, a single pair of substrate and product is considered. In the model, the substrate S is injected at a constant rate; binding of the substrate to the two states of the enzyme with different affinities results in the transformation of S into product P. The two enzyme conformations, R and T, may also differ in catalytic activity; thus the R state is either more active or has a greater affinity for the substrate. The enzyme consists of n subunits, or protomers, whose transition between the two conformations is taken as concerted. Positive feedback is accounted for by assuming that the product binds to a regulatory site on the R state and thereby shifts the

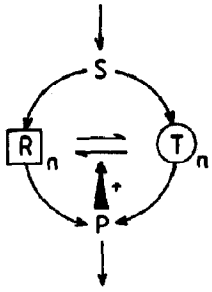

Fig. 1. Schematic model of an allosteric enzyme reaction activated by the reaction product. The substrate (S), arriving at a constant rate, binds to the two states of the enzyme, R and T, which differ in affinity for the substrate and/or catalytic activity. The enzyme contains n subunits whose transition between the two conformations is taken as concerted. The product (P) binds to the R state of the enzyme and thereby shifts the conformational equilibrium from the T to the R state. This regulation is at the core of the mechanism responsible for glycolytic oscillations (see text for details).

conformational equilibrium from the T state – which predominates in the absence of ligand – to the more reactive R state. The product leaves the system at a rate proportional to its concentration.

The dynamics of the product-activated enzyme reaction is governed by two kinetic equations for the substrate (α) and product (γ) concentrations [18,19]; these equations are obtained when assuming a quasi-steady state for the enzymatic species:

$$\frac{d\alpha}{dt} = v - \sigma\phi$$

$$\frac{d\gamma}{dt} = q\sigma\phi - k_s\gamma \qquad (1)$$

where the rate function ϕ is given by eq. 2:

$$\phi = \left\{\left[\alpha e(1+\alpha e)^{n-1}(1+\gamma)^n + L\theta\alpha ce'(1+\alpha ce')^{n-1}\right]\right\} \times \left\{\left[L(1+\alpha ce')^n + (1+\alpha e)^n(1+\gamma)^n\right]\right\}^{-1} \qquad (2)$$

In the above equations, $\alpha = [S]/K_R$ and $\gamma = [P]/K_P$, where K_R and K_P denote the dissociation constants for binding of the substrate and product to the R state; $q = K_R/K_P$; $c = K_R/K_T$ is the nonexclusive binding coefficient of the substrate, K_T being the dissociation constant related to the T state; v and σ are the substrate input and the maximum enzyme reaction rate, divided by K_R; n denotes the number of protomers; L is the allosteric constant defined [2] as the ratio of enzyme in the T and R states in the absence of ligand; θ is the ratio of catalytic constants for the T and R states (thus, $\theta = 0$ when the T state is inactive); e and e' are constants which involve the ratio of the catalytic constant and of the microscopic dissociation constant of the enzyme-substrate complexes for the R and T states (see refs 18 and 19 for further details). When e and e' are included in the normalization of α and c, and in the simple case of exclusive binding of substrate to the R state ($c = 0$), function ϕ reduces to the simpler form:

$$\phi = \frac{\alpha(1+\alpha)^{n-1}(1+\gamma)^n}{\left[L + (1+\alpha)^n(1+\gamma)^n\right]} \qquad (3)$$

Eq. 3 is similar to that obtained by Monod, Wyman and Changeux [2] in the case of exclusive binding of substrate (α) in the presence of a positive effector (γ). What is specific to the present model is that the positive effector is the product of the enzyme reaction; this creates the positive feedback loop that leads to oscillations.

The system formed by eqs 1 with ϕ defined by eq. 2 or 3 has been investigated in detail [18,19]. This system admits a single steady state which can become unstable beyond critical values of the parameters. In the phase plane (α,γ), it can be shown [20,21] that the steady state is unstable whenever it is located in a region of sufficiently negative slope ($d\alpha/d\gamma$) on the nullcline ($d\gamma/dt$) $= 0$ (see fig. 2). The fact that the latter nullcline possesses an N shape when the product concentration γ is taken as abscissa is thus of primary importance for the occurrence of oscillations. Such a shape results from the interplay between positive feedback and enzyme cooperativity.

When the steady state is unstable, it is surrounded by a stable limit cycle (dashed curve in fig. 2) which corresponds to sustained oscillations of the substrate and product concentrations as a function of time. The characteristics of limit cycle oscillations have been studied as a function of the

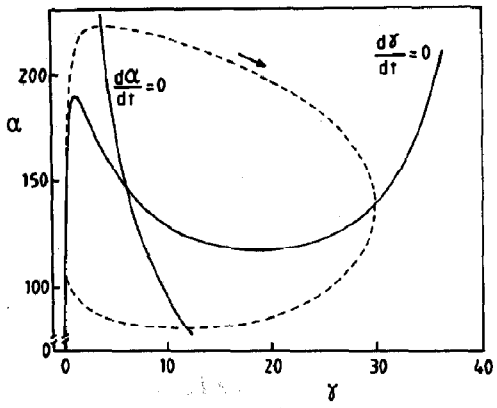

Fig. 2. Evolution towards a limit cycle (dashed line) in the product-activated allosteric enzyme reaction. Also represented in this schematic phase portrait are the substrate and product nullclines (unbroken lines). The steady state is unstable whenever it is located in a region of sufficiently negative slope on the product nullcline. This phase portrait is obtained when the enzyme contains two subunits (redrawn from ref. 26).

various parameters and compared with experimental observations [18,19]. While this model can only yield evolution to either a stable steady state or to a stable limit cycle, models in which the product-activated allosteric enzyme is coupled to a second reaction regulated in a similar manner can give rise to more complex dynamic phenomena such as aperiodic oscillations (chaos), complex periodic oscillations (bursting), or the coexistence between two simultaneously stable limit cycles (birhythmicity) [21–25]. We shall focus here on the prevalent case of simple periodic oscillations.

2.2. Role of enzyme cooperativity in the mechanism of oscillations

The two-variable system formed by eqs 1 supplemented by eq. 2 or 3 permits one to assess the role of positive feedback and cooperativity in the mechanism of oscillations. First, as to the role of regulation, it is clear from eqs 1–3 that without activation by the reaction product, oscillations will not occur. Indeed, in the absence of the feedback term $(1+\gamma)^n$ in these equations, the system can only reach a stable steady state. On the other hand, the cooperativity of allosteric interactions also plays an essential role, since the slope $(d\alpha/d\gamma)$ on the nullcline $(d\gamma/dt)=0$ in eqs 1 is never negative as it should be for instability, when the enzyme possesses a single protomer ($n=1$).

The influence of the number of protomers on metabolic oscillations has been determined [26]. To quantify the role of enzyme cooperativity in

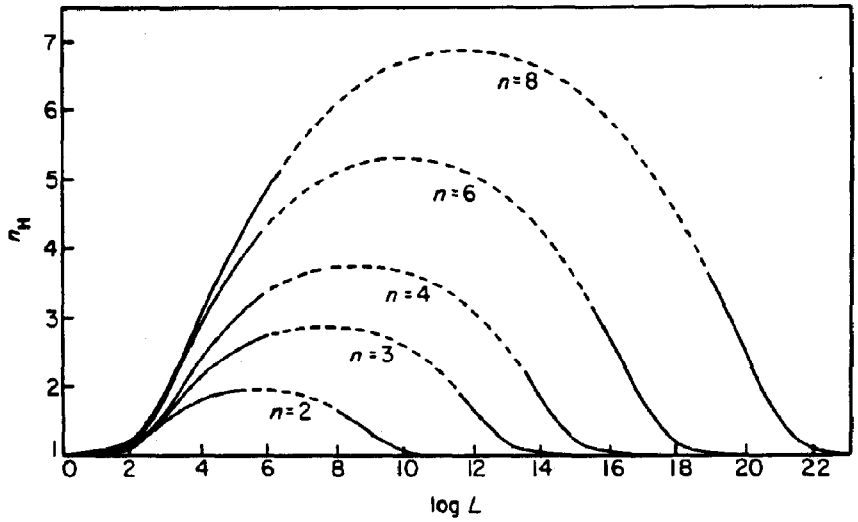

Fig. 3. Link between cooperativity and oscillations. The Hill coefficient related to the substrate is evaluated at steady state in the product-activated enzyme model for different values of the number of protomers ranging from $n=2$ to 8, as a function of the allosteric constant L. On each curve, the dashed line indicates the region of instability of the steady state (redrawn from ref. 27).

the mechanism of oscillations, the Hill coefficient n_H related to the substrate was evaluated both at steady state [27] and during the course of oscillations [28]. Shown in fig. 3 for different values of n are the curves yielding n_H as a function of the allosteric constant L in the case of a pure K system [2], i.e., when the catalytic constant is the same for the R and T states ($\theta = 1$). On each curve, the dashed line refers to instability of the steady state, determined by linear stability analysis of eqs 1. The fact that the curves have a bell shape reflects the property that cooperativity vanishes while n_H tends towards unity at both low and high values of L, when nearly all the enzyme is in the R or T state, respectively [29].

The curves of fig. 3 indicate that the domain of L values associated with instability always corresponds to the region of maximum cooperativity, given that the maximum value of n_H is close to the protomer number n in each case. Moreover, the width of the domain of instability increases with n. The results in fig. 3 also allow one to explain, in terms of a decrease in cooperativity below a critical value, the suppression of oscillations by positive as well as negative effectors [27]. An activator of the enzyme indeed decreases the effective value of the allosteric constant while an inhibitor, in contrast, produces a rise in L. In both cases, the system initially in a region of instability on the bell-shaped curves will move out of it through a decrease or increase in L.

The results obtained by correlating the Hill coefficient with instability at the steady state concur with those obtained by following the time course of the Hill coefficient during sustained oscillations. Then also, the value of n_H remains close to its maximum value of n [28]. However, in the case of two time scales, i.e., when the product evolves much more rapidly than the substrate at high values of parameters q and k, the stability analysis of the steady state nevertheless showed that oscillations can occur with a value of n_H only slightly larger than unity [27]. This result indicates that the role of cooperativity can become secondary in certain circumstances. As demonstrated below, cooperativity can in fact be totally bypassed as a requirement for sustained oscillations in the product-activated enzyme model.

2.3. Oscillations in the absence of enzyme cooperativity

Under the conditions where the system schematized in fig. 1 is governed by eqs 1, and in particular under the assumption of a linear removal rate for the reaction product, phase plane analysis indicates that oscillations cannot occur unless the number of protomers is at least equal to 2 [21,26]. As recalled in section 2.2, this result stems from the fact that no region of negative slope ($d\alpha/d\gamma$) exists on the product nullcline when $n = 1$. The question arises, however, as to what happens when the assumption of a linear sink for the product is replaced by that of a hyperbolic one corresponding to a reaction catalyzed by a Michaelian enzyme (in the domain of first-order kinetics, this case reduces to the previous situation). The system is then governed by eqs 4 where function ϕ remains defined by either eq. 2 or 3, while r_s and μ denote, respectively, the maximum rate of the sink reaction and the Michaelis constant of the sink enzyme, divided by K_P:

$$\frac{d\alpha}{dt} = v - \sigma\phi$$

$$\frac{d\gamma}{dt} = q\sigma\phi - \frac{r_s\gamma}{\mu + \gamma} \qquad (4)$$

The properties of eqs 4 resemble those of eqs 1 with regard to the occurrence of oscillatory behavior. A major difference, however, is that the steady state can now become unstable for $n = 1$, i.e., in the absence of cooperativity of the product-activated enzyme. This may happen despite the fact that in the phase plane (α,γ), the nullcline $(d\gamma/dt) = 0$ does not possess an N shape. Indeed, this would require that the equation of that curve be at least of the third degree in γ, since within a certain range of α values, three intersections of a horizontal with the nullcline, corresponding to as many distinct values of γ, should be obtained.

For $n = 1$ in eq. 3, the second expression of eq. 4 yields the following equation for the product nullcline:

$$q\sigma\phi = \frac{r_s\gamma}{\mu + \gamma}$$

or

$$\frac{\alpha(1+\gamma)}{[L+(1+\alpha)(1+\gamma)]} = \frac{r_s\gamma}{q\sigma(\mu+\gamma)} \qquad (5)$$

which is only of the second degree in γ. Although the product nullcline is no longer N-shaped, it nevertheless possesses a region of negative slope ($d\alpha/d\gamma$), as shown in fig. 4. What is lacking, with respect to the previous situation considered in fig. 2, is the ascending branch of the curve at high values of γ.

Linear stability analysis with $n = 1$ in eq. 3 shows once more that the unique steady state permitted by eqs 4 is unstable when located in a region of sufficiently negative slope on the product nullcline (see appendix A). A limit cycle surrounding the unstable steady state is again reached under these conditions. This limit cycle is peculiar in that it extends to extremely high values of γ (cf. figs 2 and 4). This is a consequence of the lack of an ascending branch on the product nullcline at high γ values: in the case of fig. 2, the maximum in γ is attained when the trajectory

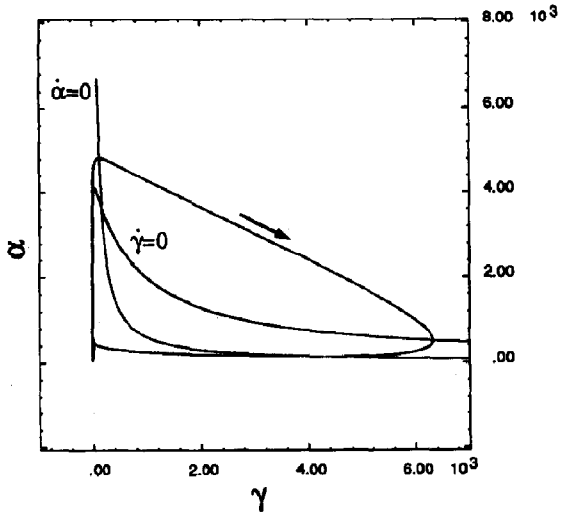

Fig. 4. Evolution towards a limit cycle in the absence of cooperativity of the product-activated enzyme, when the sink of the reaction product obeys Michaelian kinetics. The substrate and product nullclines are indicated. The trajectory is obtained by numerical integration of the kinetic equations (eqs 4) where function ϕ is defined by eq. 2 with $n = 1$. Other parameter values are: $L = 5\times10^6$, $\sigma = 10\ s^{-1}$, $e = 1.1^{-1}$, $\theta = c = 0$, $v = 1\ s^{-1}$, $q = 3$, $r_s = 12\ s^{-1}$, $\mu = 500$.

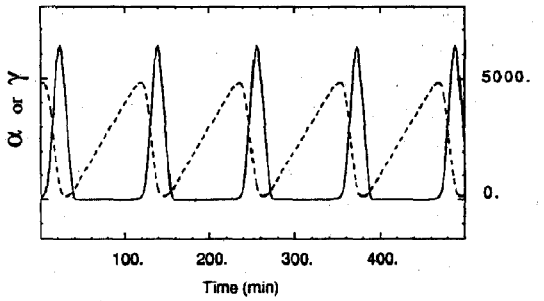

Fig. 5. Sustained oscillations corresponding to the limit cycle shown in fig. 4. The substrate (α; ---) and product (γ; ———) normalized concentrations are represented as a function of time.

followed by the system in the phase plane intersects with the right-hand limb of the product null cline; in the situation of fig. 4, in contrast, the maximum occurs at much greater values of γ, since the trajectory turns back towards the left (i.e., towards lower values of γ) only when it intersects with the part of the null cline that is nearly parallel to the horizontal axis.

The fact that the limit cycle becomes gigantic also results in a significant increase in the period of oscillations, as shown in fig. 5. That oscillations may occur for $n = 1$ does not necessarily signify that enzyme cooperativity does not play a primary role in glycolytic oscillations (see section 4). What this result does indicate is that cooperativity is not an absolute prerequisite for sustained oscillations in the reaction catalyzed by the product-activated allosteric enzyme.

3. The role of cooperativity in signal-induced calcium oscillations

We now wish to explore the role played by the cooperativity of allosteric regulation in a second oscillatory system for which the problem can be investigated systematically thanks to the existence of a theoretical model. Intracellular oscillations of Ca^{2+} have been the focus of much research in recent years. These oscillations occur in the form of a train of Ca^{2+} spikes triggered by a rise in an external signal such as a hormone or neurotransmitter. The oscillations occur in a variety of sys-

tems, from hepatocytes to endothelial cells, and from pituitary cells to oocytes, with periods ranging from less than 1 s to minutes (see refs 30–32 for recent reviews).

3.1. Model for signal-induced Ca^{2+} oscillations

The model considered for signal-induced Ca^{2+} oscillations is depicted schematically in fig. 6. It is based on the observation that a rise in the external signal is first transduced into an increase in inositol 1,4,5-trisphosphate (IP_3) within the cell; this rise in IP_3 itself triggers the release of calcium from an IP_3-sensitive store [33]. The rise in cytosolic Ca^{2+} then induces the release of Ca^{2+} from an IP_3-insensitive store, through a process of Ca^{2+}-induced Ca^{2+} release [34,35]. When taking into account the Ca^{2+} input from the external medium, the extrusion of Ca^{2+} from the cell and the pumping of Ca^{2+} into the IP_3-insensitive store, the evolution with time of the Ca^{2+} concentration in the cytosol (Z) and in the IP_3-insensitive store (Y) is governed by the following kinetic equations where parameters and concentrations are defined with respect to the total cell volume (see refs 36 and 37 for further details):

$$\frac{dZ}{dt} = v_0 + v_1\beta - v_2 + v_3 + k_f Y - kZ$$

$$\frac{dY}{dt} = v_2 - v_3 - k_f Y \qquad (6)$$

In the above equations, v_0 and kZ refer to the constant entry of Ca^{2+} within the cell and to Ca^{2+} extrusion, respectively. The saturation function of the IP_3 receptor, which possesses a cooperative nature [38], is denoted by β; this function is treated as a parameter that increases with the extent of external stimulation; $v_1\beta$ denotes the IP_3-modulated release of Ca^{2+} from the IP_3-sensitive store, which persists as long as the stimulation continues; v_2 and v_3 are the rates of Ca^{2+} pumping into and release from the IP_3-insensitive store; $k_f Y$ denotes a leaky transport of Ca^{2+} from the latter compartment into the cytosol (see fig. 6).

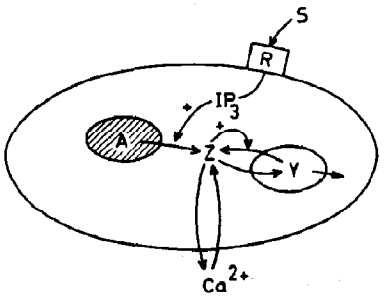

Fig. 6. Regulatory scheme for the signal-induced mobilization of Ca^{2+}, based on this Ca^{2+}-induced Ca^{2+} release from an IP_3-insensitive intracellular store. The model based on this regulatory process gives rise to repetitive Ca^{2+} transients induced by the steady rise in IP_3 triggered by the external stimulus (S) (see text and refs 36 and 37).

To take into account the activation of Ca^{2+} release from the IP_3-insensitive store by cytosolic Ca^{2+}, and to allow for cooperativity in the pumping, release and activation processes, the rates v_2 and v_3 are expressed as follows:

$$v_2 = V_{M2}\frac{Z^n}{(K_2^n + Z^n)},$$

$$v_3 = V_{M3}\frac{Y^m}{(K_R^m + Y^m)} \cdot \frac{Z^p}{(K_A^p + Z^p)} \qquad (7)$$

For the sake of simplicity, the kinetics retained is that of the Hill equation; values of n, m and p greater than unity correspond to positive cooperativity. Parameters K_2, K_R and K_A are threshold constants for the pumping, release and activation processes, while V_{M2} and V_{M3} denote the maximum rates of pumping and Ca^{2+}-activated release, respectively.

3.2. Ca^{2+} oscillations with and without cooperativity

Analysis of eqs 6 indicates that at low values of β, the system reaches a stable steady state characterized by a low level of cytosolic Ca^{2+}. Then, upon increasing external stimulation – which rise is reflected by an increase in β – sustained oscillations in cytosolic Ca^{2+} develop (see fig. 7a); these oscillations are accompanied by a saw-tooth variation of Ca^{2+} in the IP_3-insensitive intracellular store. A further increase in β leads to a shortening of the period, until the oscillations disappear when a higher, critical value of β is reached, beyond which the system reaches a stable steady state corresponding to a high level of cytosolic Ca^{2+} [36,37].

These results account well for the experimental observations, including the increase in the frequency of Ca^{2+} spiking as the level of the stimulus or the extracellular Ca^{2+} concentration rises. In contrast with an alternative theoretical model based on a feedback of Ca^{2+} on IP_3 production [39], a specific prediction of the present model is that sustained oscillations in cytosolic Ca^{2+} may occur in the absence of concomitant oscillations in IP_3. On the other hand, this model is closely related to that proposed by Kuba and Takeshita [40] for membrane potential oscillations in sympathetic neurons treated with caffeine; there also, oscillations were due to Ca^{2+} spikes resulting from the mechanism of Ca^{2+}-induced Ca^{2+} release, but the role of IP_3, which has since been elucidated, was not considered.

The results shown in fig. 8a were obtained in the case where Ca^{2+} pumping into the intracellular store, Ca^{2+} release from this compartment, and activation of this release by cytosolic Ca^{2+} are all characterized by positive cooperativity. Is such cooperativity required for oscillatory behavior? The data shown in fig. 8b indicate that this is not the case: oscillations are indeed observed in this situation for $m = n = p = 1$, i.e., when each of the three mechanisms of pumping, release and activation is described by a purely Michaelian process. The characteristics of Ca^{2+} oscillations are somewhat different from those under the conditions of fig. 8a: the period and the amplitude both increase when the three Hill coefficients are equal to unity, while the waveform is much smoother. A similar increase in amplitude is seen in the allosteric model for glycolytic oscillations for the case of decreasing or suppressing cooperativity (cf. figs 2 and 4). It should be noted, however, that the relative magnitude of the three Hill coefficients appears to be an equally important factor. Thus, when $m = n = p = 2$, the characteristics of the oscillations are very similar to those observed in fig. 8b when the three coefficients are equal to unity.

In the phase plane, the phase portrait associated with periodic behavior (fig. 7) resembles that obtained for the product-activated allosteric enzyme (figs 2 and 4); this resemblance stems from the fact that positive feedback is operative in both systems. Here also, the Z nullcline possesses a region of negative slope that is essential for insta-

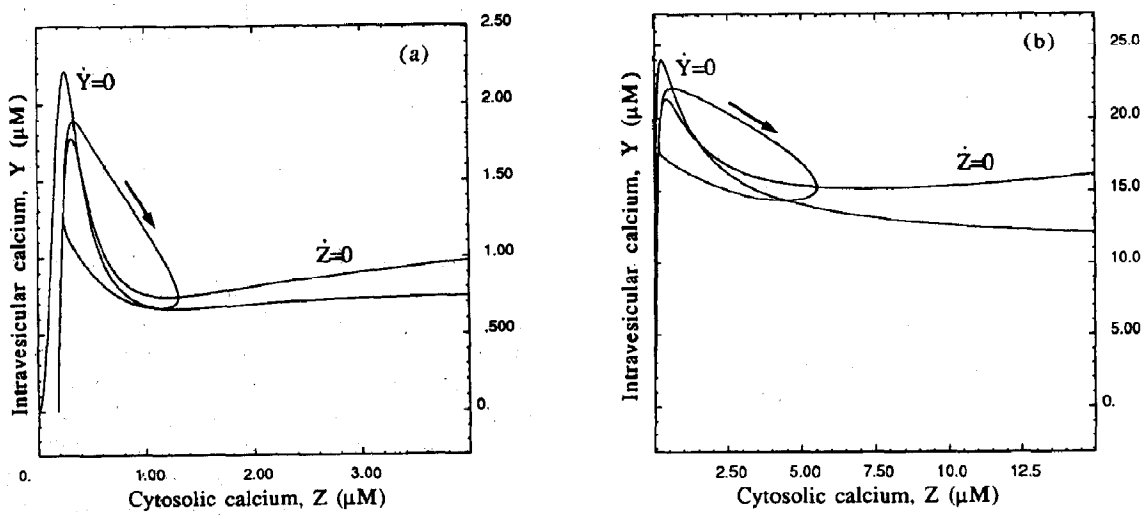

Fig. 7. Phase portrait of intracellular Ca^{2+} dynamics when Ca^{2+} pumping, Ca^{2+} release and its activation by cytosolic Ca^{2+} are cooperative (a) or not (b). In each case, the closed curve denoted by an arrow indicates the limit cycle to which the system evolves. Also represented are the nullclines for cytosolic (Z) and intravesicular (Y) Ca^{2+}. The trajectories are obtained by integration of eqs 6 and 7 for $v_0 = 1\ \mu M\ s^{-1}$, $v_1\beta = 3\ \mu M\ s^{-1}$, $K_2 = 1\ \mu M$. Other parameter values: (a) $m = n = 2$, $p = 4$, $v_2 = 65\ \mu M\ s^{-1}$, $v_3 = 500\ \mu M\ s^{-1}$, $K_R = 2\ \mu M$, $K_A = 0.9\ \mu M$, $k_f = 1\ s^{-1}$, $k = 10\ s^{-1}$; (b) $m = n = p = 1$, $v_2 = 100\ \mu M\ s^{-1}$, $v_3 = 1000\ \mu M\ s^{-1}$, $K_R = 100\ \mu M$, $K_A = 2.5\ \mu M$, $k_f = 0.1\ s^{-1}$, $k = 2\ s^{-1}$.

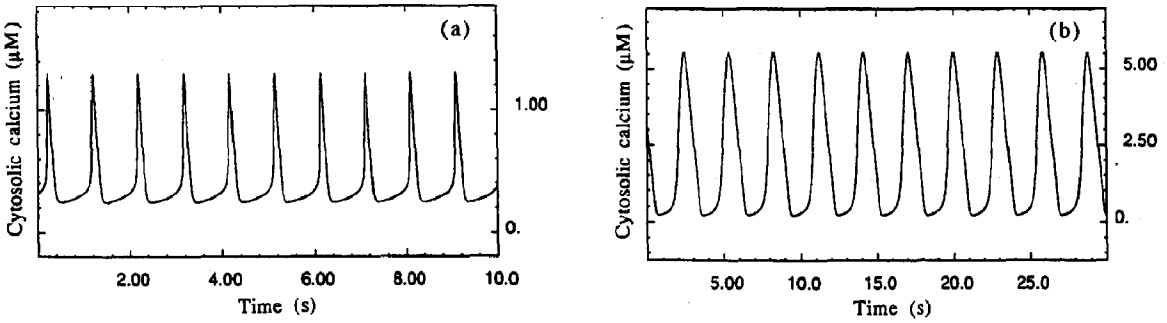

Fig. 8. Sustained oscillations of cytosolic Ca^{2+}. Panels a and b correspond to the situations described in fig. 7a and b, respectively.

bility; this region still exists when the cooperativity of the three processes of pumping, release and activation vanishes (cf. fig. 7a and b). In fact, the effects of the cooperativity coefficients n, m and p are not equivalent: an increase in the Hill coefficient n of the pumping process tends to dampen oscillatory behavior, while the converse is true for the Hill coefficients m and p which characterize release and activation. This is due to the fact that an increase in the efficiency of Ca^{2+} pumping from the cytosol into the store counteracts the activation of Ca^{2+} release by cytosolic Ca^{2+}; this activation is at the core of the instability that leads to oscillations.

If cooperativity is not absolutely required for Ca^{2+} oscillations, the same conclusion does not hold true for the feedback process. Repetitive Ca^{2+} spiking is indeed dependent on the presence of the allosteric regulation provided here by the Ca^{2+}-induced Ca^{2+} release. Such regulation might not be the only one involved in the generation of periodic behavior. Regulation of IP_3 production by Ca^{2+} is another possible source of instability [32,39]. Indeed, the very fact that complex Ca^{2+} transients in the form of bursts are sometimes observed in response to external stimulation [30–32] suggests that several instability-generating mechanisms may cooperate in producing complex periodic phenomena such as bursting or chaos [22,24]. This conjecture is supported by the analysis of extensions of the basic model of fig. 6 which allow for oscillations in IP_3 (Dupont and Goldbeter, manuscript in preparation).

3.3. Absence of Ca^{2+} oscillations in the domain of first-order kinetics

The fact that oscillations occur when the kinetic expressions for pumping, release and activation are of the Michaelian type raises the possibility that periodic behavior might also occur when each of these expressions becomes linear, since Michaelian functions reduce to linear ones in the domain of first-order kinetics. To examine this possibility, let us consider the behavior of system 6, when eqs 7 for v_2 and v_3 transform into eqs 8:

$$v_2 = k_2 Z, \; v_3 = k_3 YZ \tag{8}$$

It can be shown (see appendix B) that, under these conditions, the system of eqs 6 still admits a unique steady state, but the latter is always stable. This rules out the occurrence of sustained oscillations around a nonequilibrium unstable steady state. Such a result is consistent with previous studies of two-variable systems governed by polynomial kinetics; these studies indicated that a nonlinearity higher than quadratic is needed for limit cycle oscillations in such systems [7,41]. Thus, it is essential for the development of Ca^{2+} oscillations that the kinetics of pumping, release or activation be at least of the Michaelian type.

4. Discussion

The role of allosteric cooperativity in biochemical oscillations has been examined in two exam-

ples which relate to an enzyme reaction and to Ca^{2+} transients within the cell. While cooperativity of allosteric interactions in each case was found to favor the occurrence of oscillations by increasing the degree of nonlinearity of the kinetic equations, we have shown that it does not constitute an absolute requirement for periodic behavior in these biochemical systems. Conditions were indeed established under which sustained oscillations occur when the regulatory steps are governed by Michaelian kinetics. However, other steps characterized by the same, mild nonlinearity were needed to generate oscillations. It appears, therefore, that several Michaelian nonlinearities distributed at different reaction loci within the system may cooperate to generate the instability leading to periodic behavior.

Thus, in the allosteric model for the product-activated reaction analyzed in section 2 for glycolytic oscillations, cooperativity in the regulated enzyme is absolutely required for oscillatory behavior as long as this reaction provides the only source of nonlinearity within the system, i.e., when product removal obeys first-order kinetics. As soon as this assumption is relaxed by considering that the removal kinetics becomes Michaelian, oscillations occur even when the product-activated enzyme loses its cooperative properties by becoming monomeric. From a mathematical point of view, the degree of nonlinearity of the product nullcline drops to 2, but this is still sufficient to provide a region of negative slope that is essential for oscillations (see appendix A). This highlights the role of *diffuse nonlinearities* in the onset of oscillations: although no single step is characterized by cooperativity, moderate nonlinearities distributed over several steps combine to produce the global nonlinearity required for periodic behavior.

From a biological point of view, the importance of the removal term can be comprehended owing to the role of the activation by the reaction product as destabilizing feedback process. When the product is removed according to first-order kinetics, its effect is efficiently damped so that cooperativity of the allosteric regulation is needed to counteract the rapid loss of effector. In contrast, when the product removal becomes Michaelian, the loss of effector is buffered and, hence, less rapid as the sink saturates at high product concentrations. Then, cooperativity of activation is no longer essential and instability as well as oscillations may still occur when the allosteric enzyme becomes monomeric.

The finding that saturability of the sink relaxes the cooperativity requirement for oscillations to occur in the product-activated enzyme system parallels results obtained in studies of biochemical oscillations based on end-product inhibition and of oscillations in protein synthesis based on gene repression. Early studies showed that the degree of cooperativity of the negative feedback required for oscillations was unreasonably large, except when the metabolic chain contained several reaction steps [42–44]. However, these studies were based on the assumption that all steps other than that subjected to allosteric regulation were of the first order. When the sink of the end product is Michaelian rather than linear, it is found that the degree of cooperativity of feedback inhibition required for oscillations decreases, at a given number of steps in the pathway [45,46].

Investigation of the role of cooperativity in the occurrence of signal-induced Ca^{2+} oscillations confirmed that periodic behavior does not require positive cooperativity when several steps obey Michaelian kinetics. The characteristic feature of this type of nonlinear function is that it reaches a constant value at saturation, in contrast to first-order kinetics; the latter, as demonstrated in appendix B, does not suffice to produce the instability needed for sustained oscillatory behavior.

That cooperativity of allosteric interactions is not absolutely required for periodic behavior by no means implies, however, that it does not play an important role in the occurrence of biochemical oscillations. Whereas feedback processes can be viewed as more essential in that cooperativity alone cannot give rise to oscillations, the cooperativity of allosteric interactions provides a natural source of nonlinearity in cellular processes that favors the advent of instabilities resulting from feedback regulation. In this sense, particularly when other steps operate in the domain of first-order kinetics, cooperativity permits – or at least facilitates – the occurrence of sustained oscillations. However, it is clear that the nonlinearity arising from the cooper-

ativity of the regulated step may be replaced by the conjunction of milder, diffuse nonlinearities distributed over several steps within the system.

This conclusion is corroborated by the analysis of a model for cyclic AMP (cAMP) oscillations in *Dictyostelium* amoebae [47]. In the latter study it was also found that the positive feedback exerted by extracellular cAMP on its own synthesis within the cell is responsible for excitable and oscillatory behavior. To produce oscillations, the activation of adenylate cyclase that follows binding of extracellular cAMP to the receptor must be cooperative. However, the source of cooperativity may reside either in the activation of adenylate cyclase by the cAMP-receptor complex or in the binding of cAMP to an oligomeric receptor [47]. The determining factor, therefore, is not the particular form of cooperativity but rather the global elevation of the degree of nonlinearity of the kinetic equations that allows for full expression of the oscillatory potential of feedback regulation.

Appendix A: The product-activated allosteric enzyme: conditions for oscillations in the absence of cooperativity

The system considered is that governed by eqs 4 which hold when the product sink is described by a Michaelian expression. The equations admit a single steady state in which γ is given by eq. A1 while α is derived by solution of the second degree equation obtained by equating to zero the first expression of eqs 4:

$$\gamma_0 = \frac{q v \mu}{r_s - q v} \tag{A1}$$

Linear stability analysis of this steady state yields the characteristic expression:

$$\omega^2 + \omega\left[\sigma\left(\frac{\partial\phi}{\partial\alpha}\right)_0 - q\sigma\left(\frac{\partial\phi}{\partial\gamma}\right)_0 + \frac{r_s\mu}{(\mu+\gamma_0)^2}\right] + \sigma\left(\frac{\partial\phi}{\partial\alpha}\right)_0 \frac{r_s\mu}{(\mu+\gamma_0)^2} = 0 \tag{A2}$$

Given that the independent term is positive, the two roots ω have the same sign. Instability of the steady state requires that the real part of the roots be positive. Denoting by λ the ratio $(q\sigma/r_s)$, the instability condition takes the form:

$$\frac{\left(\frac{1}{\lambda}\right)\left(\frac{\mu}{\mu+\gamma_0}\right) - (\mu+\gamma_0)\left(\frac{\partial\phi}{\partial\gamma}\right)_0}{(\mu+\gamma_0)\left(\frac{\partial\phi}{\partial\alpha}\right)_0} < -\frac{1}{q} \tag{A3}$$

It is possible to relate this instability condition to phase plane analysis, following the procedure previously developed for the case of n protomers and a linear sink of product [20,21,26]. Thus, in the phase plane (α,γ), taking condition A3 into account, it is found that the steady state, which lies at the intersection of the nullclines $(d\alpha/dt) = 0$ and $(d\gamma/dt) = 0$, is unstable whenever condition A4 is satisfied for the slope $(d\alpha/d\gamma)$ on the product nullcline:

$$\left(\frac{d\alpha}{d\gamma}\right)_0 - \frac{1}{\lambda(\mu+\gamma_0)\left(\frac{\partial\phi}{\partial\alpha}\right)_0}\left[1 - \frac{\mu}{\mu+\gamma_0} - \lambda\phi_0\right] < -\frac{1}{q} \tag{A4}$$

The quantity within brackets is nil at steady state; the instability condition therefore reduces to inequality A5

$$\left(\frac{d\alpha}{d\gamma}\right)_0 < -\frac{1}{q} \tag{A5}$$

which is the condition previously derived [20,21,26] in the case of a linear sink.

Inequality A5 indicates that the steady state becomes unstable as soon as the slope $(d\alpha/d\gamma)$ on the nullcline $(d\gamma/dt) = 0$ in this state is less than a critical value which is negative. This requires that the two nullclines intersect in a region of sufficiently negative slope on the product nullcline. The existence of such a region of negative slope is preserved in the case of a monomeric enzyme, i.e., in the absence of cooperativity (see fig. 4).

Appendix B: Absence of Ca^{2+} oscillations in the domain of first-order kinetics

We wish to show that oscillations cannot occur when Ca^{2+} pumping, Ca^{2+} release and its activa-

tion by cytosolic Ca^{2+} are all described by first-order kinetics. Then the rates v_2 and v_3 in eqs 6 are expressed by eqs 8 and the unique steady state is given by eqs A6:

$$Z_0 = \frac{v_0 + v_1\beta}{k}, \quad Y_0 = \frac{k_2 Z_0}{k_3 Z_0 + k_f} \tag{A6}$$

The linear stability analysis of eqs 6 around this state yields a characteristic equation of the form:

$$\omega^2 - T\omega + \Delta = 0 \tag{A7}$$

In eq. A7, the trace is given by the expression:

$$\begin{aligned} T &= -(k + k_2 + k_f) + k_3(Y_0 - Z_0) \\ &= -\left\{\left[k_3^2 Z_0^2 + k_3(2k_f + k)Z_0 \right.\right. \\ &\quad \left.\left. + (k + k_2 + k_f)k_f\right]\right\}\{k_3 Z_0 + k_f\}^{-1} < 0 \end{aligned} \tag{A8}$$

Since the condition of instability is $T > 0$, the inequality A8 indicates that the steady state, eqs A6, is always stable. This rules out the occurrence of sustained oscillations of Ca^{2+} when the three processes involved in Ca^{2+} mobilization within the cell are of the first-order type.

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