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Microscopic Self-Organization of Enzymic Reactions in Small Volumes

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Using an enzymic reaction with allosteric product activation as example, we show that allosterically regulated reactions in small volumes may develop strong microscopic correlations between individual molecular reaction events. Such correlations lead to coherent synchronous activity of the enzymic molecular population which is revealed in frequent periodic spiking of the reaction.

1. Introduction

The best known examples of self-organization in chemical reactions are provided by the waves in the Belousov−Zhabotinsky reaction,[1,2] the glycolytic oscillations,[3] and the spatiotemporal patterns formed in the processes of heterogeneous catalysis.[4] Their theoretical description is based on the rate equations for concentrations of reacting species and the reaction−diffusion models. But such phenomena do not represent the only possible kind of self-organization. As shown in this paper, strong deviations from equilibrium can under certain conditions develop already at the kinetic molecular level, leading to the microscopic coherence in a reacting system.

The difference between macroscopic and microscopic self-organization can be illustrated by using an example of optical oscillations. Coherence of light, generated by lasers, results from synchronous transitions between energy levels in a large number of optically active atoms. The characteristic property of this process is the presence of strong microscopic correlations between individual photon emission events in the atoms.

An optical system can also be constructed where the intensity of the generated light would periodically vary with time, but the light would not be coherent. In this system the individual atomic emission events would not be correlated, and only their mean rate, i.e., the intensity of the generated light, would be modulated with time. Simple oscillations in the light intensity represent an example of macroscopic self-organization, whereas the coherence of individual atomic transitions, typical for the lasers, indicates the existence of microscopic self-organization in the considered system.

Analogous phenomena can be found in systems of chemical reactions. Let us suppose that a reaction results in the release of product P by molecules X. When oscillations in the reaction rate are observed, the mean number of the released product molecules varies with time. However, if we look at the individual molecular reaction events (i.e., the release of molecules P by molecules X) within a short time interval, we would find that they are statistically independent and follow the Poisson distribution. Such local equilibrium is characteristic for all macroscopic chemical self-organization phenomena.

The microscopic self-organization of a chemical reaction means that individual molecular reaction events should be strongly correlated at microscopic scales, about the duration of a single reaction event. In the considered example, it means that a large part of molecules X in the entire molecular population would synchronously release the product molecules P, and thus a spike in their concentration would be created. This coherent reactivity would be an analog of coherent light generation by lasers.

To reach microscopic self-organization, certain conditions should be satisfied. In laser generation, the fundamental role is played by the phenomenon of stimulated quantum emission, in which a photon released by an atom can increase the probability of photon emission by a different atom, provided the latter is found in the excited state. It is also essential that the photons have long free path lengths, and therefore distant correlations can be established in the system.

In this paper we consider a hypothetical enzymic reaction

$$S + E \rightarrow ES \rightarrow P, \quad [P] \rightarrow 0 \tag{1}$$

where binding of substrate S by enzyme E is allosterically activated by product P. If the substrate concentration is constant, this reaction does not exhibit macroscopic rate oscillations.[5] However, in small spatial volumes for sufficiently high intensities of the allosteric regulation periodic coherent spiking of the catalytic activity of the enzymic population in such reaction can develop. The onset of spiking represents an effect of microscopic self-organization in the considered chemical system.

In the next section we formulate the conditions under which spiking can appear. The regulatory part of the reaction should be fast. Such a reaction in large volumes would have been diffusion-controlled. When, however, the size of the volume is less than the correlation length of the reaction, correlations extend over the whole volume. It is also important that the duration of individual molecular catalytic cycles in such small volumes is much larger than the characteristic diffusion-controlled time scales, such as the mixing or the transit time for the regulatory molecules.

When these conditions are satisfied, the population of enzymes together with their regulatory molecules represents a "molecular network". A simple stochastic model of the reaction 1 in the regime of a molecular network is constructed in the third section. Investigations of this model allow us to analyze the onset of spiking and its basic properties. The paper ends with the discussion of the obtained results and their possible importance for the biochemistry of a living cell.

2. Molecular Networks

The microscopic molecular mechanism of the reaction 1 includes several stages. An enzyme molecule E binds a molecule S of the substrate, and the substrate−enzyme complex ES begins a sequence of conformational changes that ends, after some time T_1, in the release of the product molecule P. When the product molecule has left, the conformation of the enzyme

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molecule returns to the initial state. This recovery stage takes some time T_2 during which binding of a substrate molecule cannot occur. Hence, only after the time $T_0 = T_1 + T_2$, which represents the duration of a molecular catalytic cycle, the enzyme molecule can begin a new cycle. Note that thus the molecular cycle has much in common with the sequence of transitions in an excitable neuron.[6]

The initial binding of a substrate molecule is allosterically regulated. We assume that the probability of binding of a substrate by an enzyme in its resting conformation is small. It becomes, however, greatly increased if an additional regulatory molecule is bound to a different site on the surface of the enzyme, and this, by means of cooperative interactions within the enzyme macromolecule, facilitates the binding of the substrate. It is assumed that the enzyme has only one binding site for a regulatory molecule, and its binding can only take place when an enzyme is in its resting state, i.e., when the previous catalytic cycle is finished.

In the considered hypothetical reaction 1, the regulatory role is played by the product molecules, and thus the reaction is effectively autocatalytic. The product molecules decay, and therefore their lifetimes are finite. To exclude immediate triggering of a new molecular catalytic cycle by the product molecule that has just been released by the enzyme, we assume that the lifetime of a product molecule is shorter than the molecular recovery time T_2. Under this condition, the product molecules can execute their regulatory roles only by traveling through the volume and initiating the catalytic cycles of other enzymes in the population.

Since traveling of regulatory product molecules is realized by means of a random diffusion walk through the reaction volume, characteristic diffusion times are important in determining the actual kinetics of this reaction.

The mixing time t_{mix} is defined as the time after which a regulatory molecule, released at some point in the volume, can be found with equal probability anywhere inside it. If the volume has the linear size L and the diffusion constant of the regulatory molecules is D, the mixing time can be estimated, on the order of magnitude, as

$$t_{mix} = L^2/D \tag{2}$$

Another important characteristic time of the process is the traffic time $t_{traffic}$, which is defined as follows: Suppose that we have released a regulatory particle somewhere inside the volume that contains only one target enzyme molecule. Then $t_{traffic}$ represents the characteristic time after which the regulatory particle will find this enzyme molecule and bind itself to the atomic target group on its surface.

It should be noted that the last stage of this process could actually be quite complicated and involve docking by electrostatic interactions and two-dimensional diffusion of the regulatory molecule over the surface of the enzyme until the target site is reached.[7,8] In our simple treatment we neglect all these possible complications and assume that the regulatory molecule performs free Brownian motion in the volume until it touches a small target of radius R attached to the surface of the enzyme molecule. Once touching has occurred, the regulatory molecule becomes bound to the target site.

Because the molecular weight of enzymes is larger than that of the regulatory molecules, the targets can be viewed as immobile. Under these conditions, the characteristic traffic time can be estimated using the concepts of the theory of diffusion-controlled reactions.[9,10] In the order of magnitude, it is given by (cf. refs 11−13)

$$t_{traffic} = L^3/DR \tag{3}$$

where R is the radius of a target. (If the size of the atomic target group in the enzyme is comparable to the size of a regulatory molecule, it should be replaced by the sum of the two respective radii.)

Since the volume contains a population of N enzymes of the same kind, we can also introduce the transit time $t_{transit}$ that is the time after which, on the average, the regulatory molecule will find the first target (and bind to it). Assuming that targets are independently and uniformly distributed through the reaction volume, we have

$$t_{transit} = (1/N)t_{traffic} \tag{4}$$

or, explicitly,

$$t_{transit} = L^3/NDR \tag{5}$$

Note that $L_{corr} = (Dt_{transit})^{1/2}$ yields the mean distance passed by the regulatory molecule before it finds a target, and this characteristic length can be considered as the correlation radius of the reaction. This distance can be compared with the linear size L of the reaction volume.

When $L \gg L_{corr}$, the target will be found in a close proximity of the point where the regulatory product molecule has been released. Since the regulatory molecules, conveying information about the reaction events, are trapped in this case not far from the points where they have been produced, they cannot maintain long-range correlations in the reacting system.

A completely different situation is found if the condition $L \ll L_{corr}$ is satisfied. Now, the first target is found by the regulatory molecule only after it has traveled extensively through the reaction volume and has crossed it many times. It means that the regulatory molecule would be able, with equal probability, to bind any of N enzymes in the population, no matter where they are located inside the volume.

The latter condition can also be expressed in terms of the characteristic times of the diffusion problem, i.e., can be written as

$$t_{mix} \ll t_{transit} \tag{6}$$

When the transit time is much larger than the mixing time, the first target is found with equal probability anywhere in the volume.

According to eq 5, the transit time is inversely proportional to the total number N of enzyme molecules. Therefore, the condition (6) can be satisfied only for sufficiently small numbers of the enzymes. By putting $t_{mix} = t_{transit}$, we derived the estimate for the critical number of the enzyme molecules,

$$N_{cr} = L/R \tag{7}$$

Thus, it is controlled in the order of magnitude only by two parameters: the linear size L of the reaction volume and the radius R of the atomic target group on the surface of the enzyme molecule. When the number N of enzyme molecules is less than N_{cr}, the product-mediated allosteric interactions between the enzymes extend over the entire reaction volume. Note that eq 7 yields the critical enzyme concentration

$$c_{cr} = 1/L^2R \tag{8}$$

which decreases with the linear size L of the volume.

Taking $R = 1$ nm as the characteristic size of the atomic target group, we see that the critical number of enzyme molecules in the volume of size $L = 0.1$ μm is $N_{cr} = 100$, and the respective enzyme concentration is $c_{cr} \approx 10^{-4}$ M. If the diffusion constant of the product molecules is $D = 10^{-6}$ cm^2/s, we find from eqs 3−5 that the mixing time is $t_{mix} = 0.1$ ms, the traffic time is $t_{traffic} = 10$ ms, and the transit time for $N = N_{cr}$ is $t_{transit} = 0.1$ ms.

The above characteristic diffusion times can be compared with the duration T_0 of a single catalytic molecular cycle. This time has not been directly measured, but some estimates of its magnitude could be obtained by measuring the maximal turnover rate of an enzymic reaction. It is believed that this rate, reached under the condition of the substrate saturation, is controlled only by the molecular turnover.[8] Hence, T_0 can be roughly estimated as the inverse of the maximal turnover rate. Thus derived, the estimates for T_0 may range, depending on a particular enzyme, from as small as fractions of a microsecond to several seconds.[14] However, the fairly typical value would be about $T_0 = 10$ ms.

Hence, for the reactions in submicrometer volumes involving hundreds of enzyme molecules, this time is much larger than both the mixing and the transit times in the reaction volume,

$$T_0 \gg t_{transit}, t_{mix} \tag{9}$$

This is a remarkable result. In a macroscopic system, all characteristic kinetic times of a chemical reaction are usually much longer than the duration of a single molecular reaction event, and therefore these events can be treated as instantaneous. This forms the basis of the traditional kinetic theory formulated in terms of the Markov random processes. We see, however, that in very small spatial volumes for enzymic chemical reactions the opposite limit may be realized, so that the characteristic times of internal molecular dynamics of the enzymes become larger than the kinetic times of the reaction. When this occurs, the theoretical description of a chemical reaction should be essentially modified.

The considered system can be viewed as a population of active macromolecules that operate like molecular machines. Each internal molecular cycle of an enzyme results in the conversion of a substrate molecule into a molecule of the product. The cycle represent a sequence of conformation changes that can be understood as continuous motion along an internal reaction coordinate. For allosteric enzymes, the molecular cycles can be externally controlled by binding of regulatory molecules. Generally, arrival of a regulatory molecule can lead either to acceleration or slowing down at some stages of a molecular cycle.

When regulatory molecules are produced by enzymes in the same population, this leads to interactions (or communication) between the cycles of different enzyme molecules. If condition (9) holds, the characteristic transport times of regulatory molecules between the enzymes are short as compared with the molecular cycle duration. Furthermore, if the transit time is larger than the mixing time in the volume, a regulatory molecule released by a given enzyme can influence, with equal probability, the catalytic cycle of any other enzyme in the population. We have earlier suggested[13,15] that such interacting populations of active macromolecules should be called *molecular networks*.

In the presence of strong allosteric interactions, the internal dynamics of individual enzyme molecules in such a network can be so significantly influenced by the dynamics of other enzymes that the whole system would perform coherent collective evolution in the multidimensional phase space of internal reaction coordinates, i.e., behave as a single dynamical object.

This coherence would be manifested in rigid correlations between the individual catalytic cycles and the moments when the product molecules are released by different enzymes. It means that the catalytic activity of the network would represent a sequence of sharp spikes, each corresponding to synchronous firing of the product by a large fraction of enzyme molecules.

3. Stochastic Automata

To illustrate the general analysis of molecular networks, we consider a simple stochastic model.[16] In this model each enzyme molecule is treated as an automaton that performs a sequence of transitions between its internal states. The states of this automaton are described by an integer phase variable ϕ which takes values between 0 and K_0. The value $\phi = 0$ corresponds to the rest state of the enzyme, where it is ready to bind a substrate molecule; $\phi = 1$ is the first state of the substrate−enzyme complex. Firing of the product molecule occurs at the state $\phi = K_1$; the next K_2 states until $\phi = K_0$ correspond to the enzyme's recovery ($K_0 = K_1 + K_2$). From the state $\phi = K_0$ the enzyme goes to the rest state $\phi = 0$.

Transitions can occur only at discrete time moments $t_n = n\Delta t$, $n = 1, 2, 3, ...$ The transition from $\phi = 0$ to $\phi = 1$, i.e., binding of a substrate molecule by an enzyme, is stochastic and characterized by a certain probability w. The subsequent motion inside the cycle is deterministic: at each next time moment $n + 1$ the phase ϕ is increased by one until the state $\phi = K_0$ is reached.

The population consists of N enzymes; the phase variables of different enzyme molecules are denoted as ϕ_i, with $i = 1, 2, ..., N$. The phase dynamics is described by the algorithm

$$\phi_i(n+1) = \begin{cases} \phi_i(n) + 1, & \text{if } 0 < \phi_i(n) < K_0 \\ 0, & \text{if } \phi_i(n) = K_0 \\ 1, & \text{with probability } w, \text{ if } \phi_i(n) = 0 \\ 0, & \text{with probability } 1- w, \text{ if } \phi_i(n) = 0 \end{cases} \tag{10}$$

Whenever an enzyme passes through the state $\phi_i(n) = K_1$, a new product molecule is created. On the other hand, if the decay rate of the product molecules per unit time step is g, any product molecule can die with the probability g at each step. If $m(n)$ is the number of product molecules in the system at moment n, their number at the next moment $n + 1$ is therefore given by

$$m(n+1) = m'(n) - k \tag{11}$$

where

$$m'(n) = m(n) + \sum_{i=1}^{N} \Delta(\phi_i(n) - K_1) \tag{12}$$

takes into account new product molecules that has been released by the enzymes and k is the number of the product molecules that have died. Since each product molecule can die per unit time step with the probability g, the probability $\pi(k|m')$ that k out of $m'(n)$ molecules would die is given by the binomial distribution

$$\pi(k|m') = \frac{m'!}{k!\,(m'-k)!}\, g^k (1-g)^{m'-k} \tag{13}$$

Equations 11−13 give the stochastic algorithm for the product molecules in the system.

We must also include into the model the effects of allosteric activation by the product molecules. Assuming that the

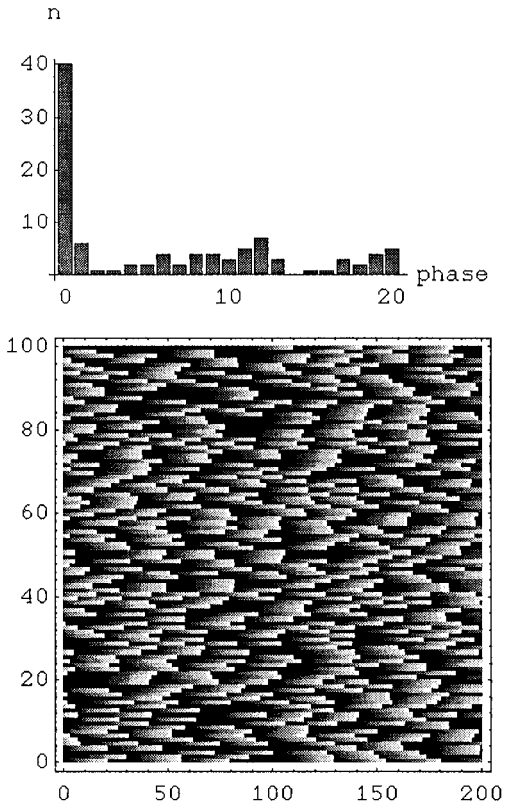

Figure 1. Molecular chaos. The histogram of the distribution of the enzymes over the cycle phases at a fixed time (a, top) and the evolution of the states of all 100 enzymes during 200 subsequent time steps (b, bottom); $K_0 = 20$, $K_1 = 11$, $w_0 = 0.01$, $w_1 = 0.01$, and $g = 0.3$.

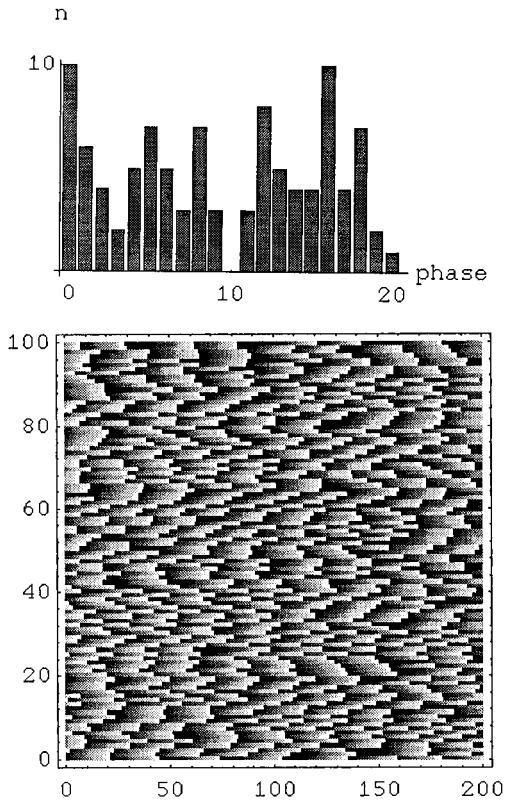

Figure 2. Enhanced fluctuations ($w_1 = 0.03$ and $K_1 = 11$, other parameters are the same as in Figure 1).

conditions

$$T_0 \gg t_{\text{transit}} \gg t_{\text{mix}} \tag{14}$$

are satisfied for the considered reaction, each of the product molecules in the volume can activate with equal probability the catalytic cycle of any given enzyme.

Let w_0 be the probability of the spontaneous cycle initiation, i.e., the probability that, per one time step, the enzyme in the rest state $\phi = 0$ binds a substrate molecule and hence a transition to $\phi = 1$ occurs. Suppose that if only one product molecule is present inside the volume, there is a probability w_1 per one time step that this product molecule would activate the cycle, i.e., produce a transition from $\phi = 0$ to $\phi = 1$. Thus, the parameter w_1 characterizes in this model the intensity of the allosteric activation.

If the volume contains m product molecules, the total probability w of cycle initiation in a given enzyme per one time step can be found by first calculating the probability q that the initiation *does not* occur, and the enzyme remains in the rest state. Since this can take place only if the spontaneous initiation has not occurred and any of m product molecules has not activated the cycle, we can write

$$q = (1 - w_0)(1 - w_1)^m \tag{15}$$

Because the probability of the cycle initiation is $w = 1 - q$, we have at moment n

$$w = 1 - (1 - w_0)(1 - w_1)^{m(n)} \tag{16}$$

This completes the definition of the stochastic automaton model of the reaction 1 in the regime of a molecular network.

In the simulations of the stochastic reaction model, we take $T_0 = 20$ ms and choose $\Delta t = 1$ ms, so that the total number of phase steps in the cycle is $K_0 = 20$. The system consists of $N = 100$ enzyme molecules, and the probability of spontaneous cycle initiation is $w_0 = 0.01$ ms^{-1}. The decay rate of the product molecules is chosen to be $g = 0.3$ ms^{-1}, so that their mean lifetime is significantly shorter than the duration T_0 of the cycle. The parameters w_1, characterizing the intensity of allosteric regulation, and $K_1 = T_1/\Delta t$, specifying the phase inside the cycle where the product molecule is released, are varied.

4. From Molecular Chaos to Coherent Spiking

By repeatedly applying the stochastic algorithm described in the previous section, the evolution of the reaction system is reproduced and can be investigated. In the first series of simulations we fix $K_1 = 11$ (note that thus the product is released in the middle of the cycle of the total duration $K_0 = 20$) and study how the reaction properties are changed as the intensity of allosteric regulation, characterized by the parameter w_1 in the model, is gradually increased.

As the initial condition in our simulations, we have always chosen a state with random distribution over the cycle phases and a small number of the product molecules. To eliminate the transient effects, the iterations have typically been continued for several thousands of time steps before the kinetic properties of the reaction have been analyzed.

Figure 1a shows the histogram of the distribution of enzymes over cycle phases at a fixed moment for the situation with weak allosteric regulation ($w_1 = 0.01$). We see that about half of the enzyme population is found in the rest state ($\phi = 0$), waiting to bind a substrate. The remaining enzymes are uniformly, though with some fluctuations, distributed over the cycle phases. The cycles of individual enzymes are not correlated. This is clearly

seen in Figure 1b that shows the cycles of all enzymes in the population.

Each horizontal stripe in Figure 1b presents subsequent phases ϕ_i of a certain enzyme i as a function of time. The gray scale representation is used to display the phase values, with the black pixels corresponding to $\phi = 0$ and the bright pixels indicating the maximal possible phase $\phi = 20$. The vertical coordinate gives the number i assigned to an enzyme in the population. Note that, under the conditions of a molecular network, each enzyme may interact with equal probability with any other enzyme in the population, and hence the enumeration order is arbitrary and irrelevant.

The reaction regime in Figure 1b has no significant correlation between the cycles of individual enzymes, so that their phases are random. It can therefore be classified as the state of molecular chaos.

We increase now the intensity of allosteric regulation to $w_1 = 0.03$. This has a strong effect on the reaction conditions. As seen in Figure 2a, the occupation number of the state with $\phi = 0$ is not much larger than the occupations numbers of others phase states. Therefore, the enzymes do not long wait now before they bind a substrate molecule, and the cycle is initiated. Since the probability w_0 of the nonactivated, spontaneous cycle initiation is the same here as in Figure 1a, this implies that the cycles are already to a large extent allosterically activated. The fluctuations in the phase distribution in Figure 2a are enhanced (and bursts of spiking occasionally occur). However, no permanent temporal order can yet be discerned in the system's behavior (Figure 2b).

When the intensity of allosteric regulation is further increased to $w_1 = 0.05$, a qualitative change in the system's behavior occurs (Figure 3a). The enzymic population breaks now into two approximately equal groups. All enzymes belonging to the same group have close cycle phases, and the shift of half the cycle period is found between the mean phases of these two groups.

Though the histogram in Figure 3a is given only for one moment, it is fairly typical. As time goes on, two enzyme groups oscillating with the phase shift of a half a period persist in the system (Figure 3b). Occasionally, an enzyme may change its allocation and move to the other group. Moreover, the phases of enzymes inside a given group display some statistical fluctuations.

Apparently, this kinetic regime is different from the state with random, uncorrelated reaction events as shown in Figure 1. It is characterized by the presence of strong correlations between microscopic cycles of individual molecules in the enzymic population. Since the product molecules are released when enzymes pass through a fixed phase state, the product generation rate would show in this case sharp narrow spikes (see also the discussion below). The width of each spike is determined by the phase variation within an enzymic group, and the time period, separating two subsequent spikes, is half of the duration of a single molecular cycle T_0. Thus, coherent microscopic spiking in the enzymic activity is the characteristic property of such microscopically organized reaction regime.

To understand why two enzyme groups become formed in the system, we should recall that, in the above series of simulations, the product molecule is released at about the middle of the reaction cycle (i.e., $T_0 \approx 2T_1$). Because the lifetimes of the product molecules are much shorter than the cycle duration, synchronous oscillations of the entire population (formation of single phase group) are not here possible (indeed, the product molecules released in a spike would then have died before the enzymes return to their rest states and can be again activated).

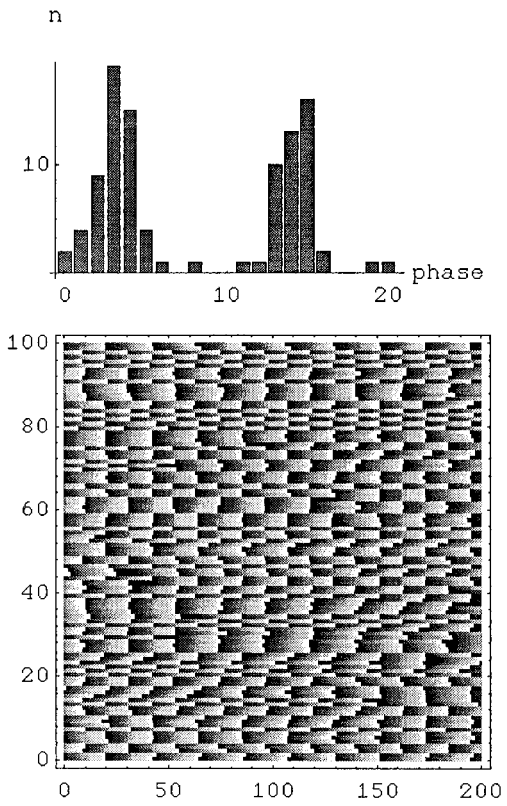

Figure 3. Spiking with two enzymic groups ($w_1 = 0.05$ and $K_1 = 11$).

However, the enzymes may still break into phase groups with the shift of half the oscillation period between them. In this case, which is realized for the simulation in Figure 3, the product molecules released by enzymes of the first group activate the cycles of the enzymes belonging to the second group, and vice versa.

These arguments indicate that the pattern of microscopic self-organization in the considered reaction may depend on the relationship between T_1 and T_0 (i.e., between $K_1 = T_1/\Delta t$ and $K_0 = T_0/\Delta t$). Therefore, in the second simulation series the parameter K_1 has been varied.

The simulations have revealed the existence of spiking regimes with a larger number of the enzymic groups. Figure 4 shows an example of spiking with three groups of enzymes that has been observed at $K_1 = 15$. Because the phases of these three groups are shifted by a third of the cycle period, they form a loop where each group activates the cycle of the next one. Since the number of the enzymes belonging to a group is now smaller, they release less product molecules in each spike, and therefore, higher intensity of allosteric regulation is needed for the onset of spiking.

The synchronous activity of the entire population (i.e., spiking with only one group) has been observed for $K_1 = 19$. In this case the recovery time is shorter than the lifetime of the product molecules, and therefore the products released by the same group can again activate it.

It is interesting that the synchronous activity has also been found (Figure 5) in the situation when the product molecules are released at an early stage of the cycle, i.e., for $K_1 = 3$. This effect has a different explanation. As seen in Figure 5a, the phases of the enzymes inside the group now significantly vary. When the "precursor" part of the group passes through the phase stage $\phi = K_1$, it releases the product molecules that activate the cycles in the central part of the enzymic group. Note that the synchronization threshold ($w_1 = 0.15$) is then larger than for the transition to spiking with two groups (cf. Figure 1).

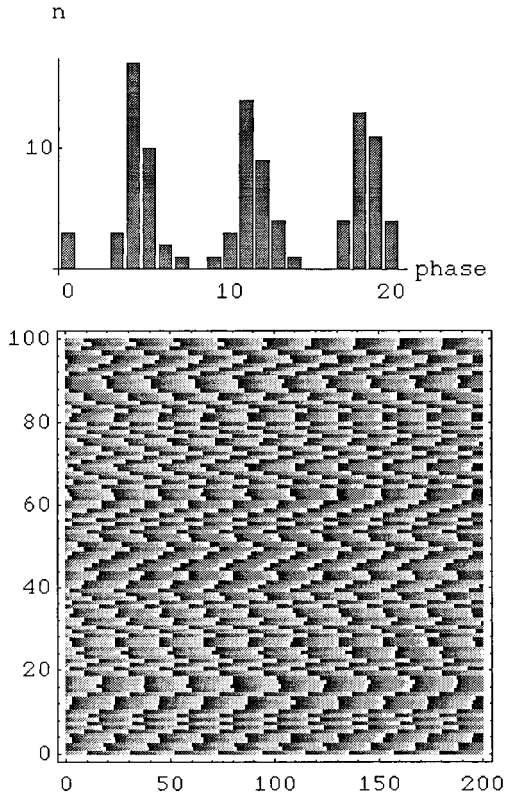

Figure 4. Spiking with three enzymic groups ($w_1 = 0.15$ and $K_1 = 15$).

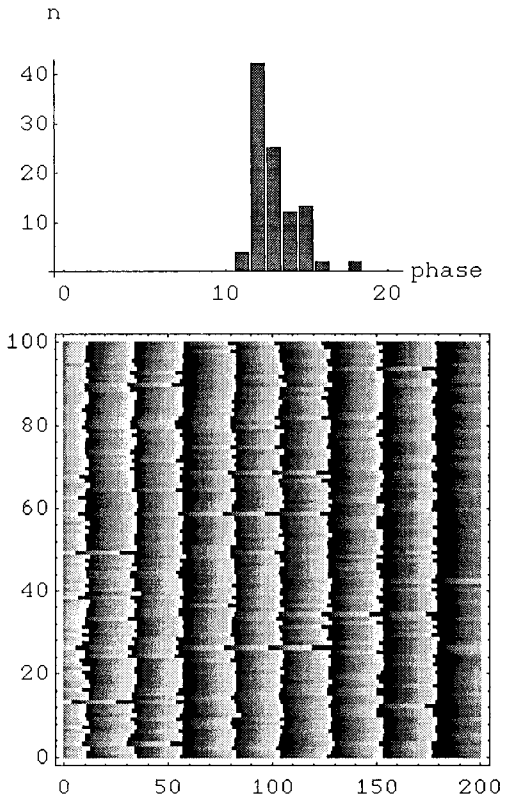

Figure 5. Synchronous spiking of the entire enzymic population ($w_1 = 0.15$ and $K_1 = 3$).

During the transient leading from the initial distribution with random phases to the stable spiking regime with a certain number of groups, a larger number of enzyme groups is first formed. However, such additional groups later disappear by a process which looks like competition between the groups. For some parameter values, the intermittent behavior has been observed: the bursts of coherent spiking alternated with the periods of apparently chaotic activity.

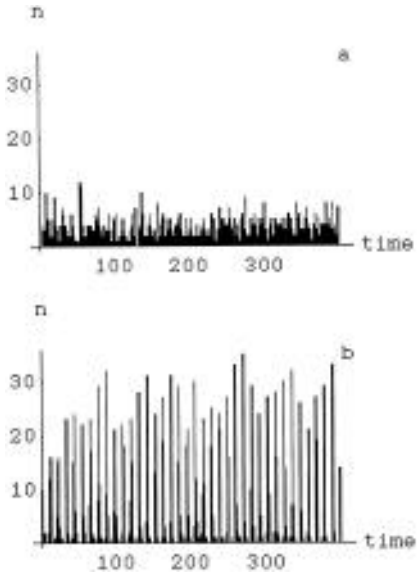

Figure 6. Time-dependent generation rate of the product molecules (a) in the regime of molecular chaos ($w_1 = 0.01$ and $K_1 = 11$) and (b) for the spiking with two enzymic groups ($w_1 = 0.07$ and $K_1 = 11$).

An important property characterizing the kinetics of an enzymatic reaction is the time-dependent product generation rate, i.e., the number of new product molecules appearing at each time step. When the molecular cycles of individual enzymes in the population are not correlated, this rate randomly fluctuates around a certain mean level (Figure 6a). The intensity of such fluctuations agrees with that predicted by the Poissonian statistics.

In contrast to this, when strong correlations between individual molecular cycles are present, the product is released only in short narrow spikes separated by time intervals where the entire enzymic population is practically silent (Figure 6b). Each spike is produced by action of the enzymes in a certain group. The interval between two subsequent spikes is therefore equal to the duration of a single molecular cycle divided by the number of the synchronous enzymic groups in the system. Note that random phase variations within an enzymic group lead to the fluctuations in the amplitudes of the spikes that are seen in Figure 6b.

5. Discussion and Conclusions

A living biological cell is a tiny chemical reactor where tens of thousands of chemical reactions can simultaneously go on. The very fact that these reactions proceed in a regular and predictable manner, and properly respond to variations in environmental conditions, already indicates a high degree of organization in this system.

The biochemical activity of a cell can be compared with the operation of a large industrial factory or an assembly line where certain parts are produced by a system of machines. The products of one machine are then used by other machines for manufacturing of their products.

Two possible general modes of operation of such a factory can be imagined. In the asynchronous mode, the parts produced by a given machine are first deposited in a common store and then taken from the store by other machines, when these parts are needed for further production. This kind of the organization is not however optimal, since it requires large storage facilities and many transactions. It becomes deficient when the inter-

mediate products are unstable and can easily be damaged or destroyed during the storage process.

When the synchronous operation mode is employed, the intermediate products, required for a certain operation step in a given machine, are released by other machines and become available exactly at the moment when they are needed. Hence, large storage facilities can be eliminated, and the entire production process may run much faster. Apparently, such synchronous manufacturing process implies much more complicated management than the asynchronous operation mode. In a real factory, this is achieved by careful planning and external control of the production.

For a living cell, the role of the "machines" is played by individual enzyme molecules. The asynchronous mode of operation corresponds here to the usual kinetic regime of a chemical reaction, where intermediate products or regulatory molecules are stored in the solution. The synchronous mode would represent a kinetic regime with strong temporal correlations between the individual molecular cycles of different enzymes. An example of such behavior is provided by the phenomenon of coherent spiking, which has been considered in this paper.

Remarkably, we have found that as the strength of the allosteric regulation is increased, the synchronous kinetic regime can spontaneously emerge in the reacting system, and despite the stochastic nature of the process, no external control is necessary to maintain this mode. This is a clear effect of self-organization, which is however different from the macroscopic phenomena of pattern formation in systems with chemical reactions.

Our simple estimates have been performed for very small volumes, which are characteristic of the intracellular compartments. For the larger volumes, the mixing and traffic times rapidly increase and become larger than the duration of a molecular catalytic cycle. Therefore, similar synchronous activity of the reaction in the volume of the micrometer size would be possible only for the enzymes with the slower turnover rates, of about 1 Hz. The critical number of the enzymes in such a volume is then about 1000, which corresponds to the concentration of 10^{-6} M.

The analysis given in this paper is only a first step toward the detailed examination of microscopic self-organization in biochemical reactions. This must be followed by the detailed analysis of concrete reactions and particular mechanisms of the allosteric regulation.

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