Mutual Synchronization of Molecular Turnover Cycles in Allosteric Enzymes III. Intramolecular Cooperativity

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In small micrometer volumes typical of living cells, regulatory molecules of enzymic reactions can diffuse so fast from one enzyme molecule to another that the diffusion time is much shorter than the turnover time of an enzyme. Under these conditions, a special kinetic regime of a molecular network is realized. We consider molecular networks formed by allosteric enzymes with several functional subunits, interactions between which are described by a sequential model of Koshland et al. with heterotropic or homotropic (positive and negative) cooperativities. Simple product-activated and product-inhibited reactions are investigated. We show that allosteric cross-molecular regulation and intramolecular cooperativity result in the development of coherent dynamics of enzyme molecules in such networks. Intermolecular synchronization of turnover cycles of different enzymes is accompanied by intramolecular synchronization of the dynamics of subunits belonging to the same molecules. Strong intramolecular correlations persist even at high intensity of thermal molecular fluctuations, when intermolecular synchronization is already absent.

Introduction

The classical enzyme kinetics leading to the Michaelis−Menten law was based on the picture of discrete kinetical transitions (or reaction events), which had no duration of their own and were completely characterized by their probability rates. Hence, the description of chemical processes involving enzyme macromolecules neglected their complex intramolecular dynamics. Recent progress in theoretical understanding of protein dynamics has brought essential corrections to this simplified picture. Protein molecules are characterized by their energy landscape and, depending on the spatial arrangements of atoms and atomic groups, the same macromolecules can be found in various conformations with different energies and functional properties.[1] This has a strong impact on various aspects of the behavior of enzymes.

To achieve their native conformation, enzymes have to fold correctly. The folding process of a protein consists of a set of pathways down a folding funnel.[2,3] The width of the funnel is given by the entropy of the folding protein characterized by the number of thermally available states. The depth represents the free energy of the protein. As the folding protein proceeds down the energy surface, both entropy and free energy decrease so that the protein ends up in its native ground state. Protein folding can be looked upon as a process of complex conformational relaxation. The energy landscape is rugged because amino acid residues compete for the available space in order to minimize their energies, and there are both attractive and repulsive interactions among the monomers, which all have to be satisfied. The motion down the energy surface of the folding funnel along the reaction coordinate can be described as a drift motion superimposed by a stochastic diffusion component.[4] The free energy landscape also plays an important role in the description of the action of an enzyme, because turnover cycles of an enzyme involve conformational changes and slow conformational relaxation, which can be looked upon as a pathway on the energy surface. The energy landscape of an enzyme can be explored computationally by starting with a given conformation and then using molecular dynamics simulation to pursue how other conformations are reached. The drawback of this method is that extremely long computer runs are necessary so that simulations spanning more than 10 nanoseconds are not possible today.

Due to advances in experimental methods, it has become possible to study the operation of individual enzyme molecules. Such experiments have been performed with single molecules of β-D-galactosidase from *Escherichia coli*,[5,6] L-lactate dehydrogenase from human erythrocytes (LDH-1),[7] alkaline phosphatase from both calf intestinum[8] and *E. coli*,[9,10] horseradish peroxidase,[11] and cholesterol oxidase from *Brevibacterium* sp.[12] Further, the actin/myosin molecular motor system has been studied.[13−15] It has also been shown for the photosensitive cytochrome P-450 system that the turnover cycles of enzymes can be synchronized by subjecting enzyme molecules to external forcing by periodic light pulses.[16−18] Similar synchronization phenomena have been investigated theoretically for the membrane-located enzyme Na,K-ATPase, assuming external forcing by electrical fields.[19] Moreover, observations of coherent movement of groups of atoms within a single protein molecule have recently been reported.[20]

Our previous theoretical analysis has revealed that mutual synchronization of enzymic reaction cycles is possible in small volumes typical of living cells. In such microscopic volumes, the diffusional transport and mixing times of the substrate,

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product, and regulatory molecules of enzymic reactions are shorter than the time needed to complete a single catalytic cycle of an enzyme molecule.[21,22] So far we have analyzed the phenomenon of mutual synchronization of enzymic turnover cycles for enzymatic reactions with allosteric product activation,[23−26] allosteric product inhibition,[27] and closed reaction loops.[28]

In the present paper we want to extend our investigations to enzymes composed of several subunits. Depending on how many regulatory molecules are bound, the subunits can interact with each other, their interacting leading to the phenomenon of cooperativity. In this case, also synchronization of reaction cycles of the whole set of enzymes is to be expected, in addition to synchronization of reaction cycles between the subunits within an enzyme.

In the first section we present a stochastic model of the considered hypothetical enzymic reaction. The results of numerical simulations of this model with respect to intermolecular and intramolecular synchronization of enzymic turnover cycles are given in section 2, applying various models of cooperativity. Additionally, the noise dependence of synchronicity is investigated. The mean-field approximation of our system is constructed in section 3. At the end we summarize and discuss the results of our theoretical study. The Appendix contains the detailed algorithms used in our numerical simulations of the stochastic model.

1. Stochastic Model of Enzymes with Several Cooperating Subunits

As an approach to model the complex intramolecular dynamics of an enzyme, we have initially used discrete stochastic automata to simulate the dynamic process of an enzymic turnover cycle.[24] Later the model was extended to a continuous description of the process as a one-dimensional drift motion along a reaction coordinate.[25] The interaction of substrate and product molecules with the enzyme is also treated stochastically.

For each subunit k of enzyme i we introduce a binary variable s_{ik}, which takes a value of $s_{ik} = 0$ if the subunit under consideration is in the ground state, waiting to bind a substrate molecule. Once the enzyme−substrate complex is formed by binding a substrate molecule, the binary variable switches to $s_{ik} = 1$. The enzyme subunit now undergoes the reaction cycle described by a one-dimensional drift motion along the reaction coordinate ϕ_{ik} starting at $\phi_{ik} = 0$ and ending at $\phi_{ik} = 1$. At a certain point ϕ_p in between, the product molecule is released, and the enzyme subunit returns to the ground state through a process of conformational relaxation (see refs 29 and 30).

The evolution of the probability distribution $p(\phi_{ik},t)$ over the coordinate ϕ_{ik} satisfies the diffusional drift equation

$$\frac{\partial p(\phi_{ik},t)}{\partial t} = -v\frac{\partial p(\phi_{ik},t)}{\partial \phi_{ik}} + \sigma\frac{\partial^2 p(\phi_{ik},t)}{\partial \phi_{ik}^{\,2}} \qquad (1)$$

In this equation the drift motion is described by the first term of the right-hand side, the superimposed diffusion due to internal thermal fluctuations by the second term. Equivalenty, the same stochastic process is described by the Langevin equation

$$\frac{\mathrm{d}\phi_{ik}}{\mathrm{d}t} = v + \eta_{ik}(t) \qquad (2)$$

Here v is the drift velocity and η_{ik} is the white Gaussian noise with intensity σ and the correlation function

$$\langle \eta_{ik}(t)\, \eta_{jl}(t')\rangle = 2\sigma\delta_{ik,jl}\delta(t-t') \qquad (3)$$

In order to keep our model simple, we assume that, after having bound a substrate molecule, the enzyme moves down with constant drift velocity v the energy surface of constant negative slope. There is only one path on the energy surface possible so that the turnover time $\tau = 1/v$ of the enzymic turnover cycle and the time moment of product release $\tau_1 = \phi_p/v$ would have been constant if noise were absent. As there is a noise term added to the drift term in eq 2, the motion inside the turnover cycle has a stochastic component, and the turnover time fluctuates around its mean value for different realizations of the process. Estimates of the mean turnover time $\langle\tau\rangle$ and its statistical dispersion $\Delta\tau = \sqrt{\langle\tau^2\rangle - \langle\tau\rangle}$ are[25]

$$\langle\tau\rangle = \frac{1}{v} - \frac{\sigma}{v^2}[1 - \exp(-v/\sigma)] \qquad (4)$$

$$\Delta\tau^2 = \frac{2\sigma}{v^3} - \frac{5\sigma^2}{v^4} + \frac{4\sigma}{v^3}\exp(-v/\sigma) + \frac{4\sigma^2}{v^4}\exp(-v/\sigma) + \frac{\sigma^2}{v^4}\exp(-2v/\sigma) \qquad (5)$$

For small noise intensities σ, $\langle\tau\rangle$, $\Delta\tau$, and the relative statistical dispersion $\xi = \Delta\tau/\langle\tau\rangle$ can be approximated by $\langle\tau\rangle \approx 1/v$, $\Delta\tau \approx \sqrt{2\sigma/v^3}$ and $\xi \approx \sqrt{2\sigma/v}$. Note that the intensity of intramolecular fluctuations is the same for both subunits, and these fluctuations are not correlated in our model.

In our previous publications on synchronization of enzymic turnover cycles for enzymes activated[25] or inhibited[27] allosterically by product molecules, the enzymes were treated as single functional units. However, real enzymes are often composed of several subunits. The activity of the whole complex depends on the number of regulatory molecules bound to the binding site on each subunit. This can be achieved in several ways. Communication between subunits is mediated by conformational changes. For homotropic cooperativity, binding of a ligand influences the binding of ligand molecules of the same type to other subunits. In the case of heterotropic cooperativity, binding of ligands influences the binding of molecules of a different type, e.g., of substrate molecules. There have been developed two theoretical models of cooperativity, i.e., the sequential model[31] and the concerted model.[32] Our analysis in this paper will be performed in the framework of the sequential model of Koshland et al.[31]

(a) Heterotropic Cooperativity. To keep the model simple, we assume that enzyme molecules consist of only two subunits. Each subunit has a binding site for a regulatory molecule and a binding site for a substrate molecule. The binding of regulatory molecules is formally expressed by a binary variable r_{ij}, which has a value of 1 if a regulatory molecule has bound to subunit k of enzyme i; r_{ik} is equal to 0 if the regulatory site of subunit k of enzyme i is empty. The probability rate ν_{ik} of binding a substrate molecule to subunit k of enzyme i depends on r_{ik}. Bound regulatory molecules can dissociate at rate κ. It is convenient to introduce two constants: χ_1, describing the strength of cooperativity, and χ_2, being a measure of the strength of allosteric regulation. A scheme of our model of heterotropic cooperativity can be seen in Figure 1.

If the enzyme is *activated* by the binding of regulatory product molecules in the sequential model, the probability rate ν_{ij} of binding a substrate molecule is $\nu_{ik}(1,1) = \nu$ if both

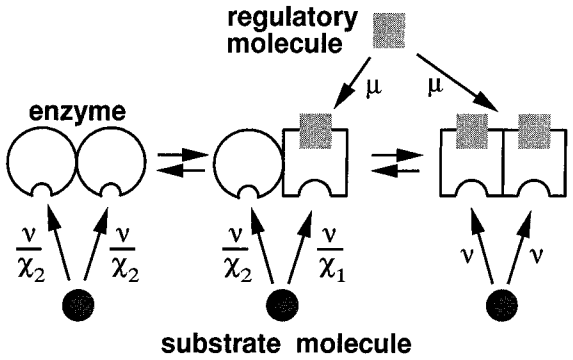

Figure 1. Model of heterotropic cooperativity for the product-activated reaction.

regulatory sites are occupied. The rate $\nu = \alpha S$ is proportional to the constant number of substrate molecules S in the system. If only the regulatory site of a given subunit is occupied and the other site is vacant, the binding rate for the respective subunit is $\nu_{ik}(1,0) = \nu/\chi_1$. If the regulatory site of the subunit is vacant, but the regulatory site of the other subunit is occupied, then $\nu_{ik}(0,1) = \nu/\chi_2$. Finally, if both regulatory sites are vacant, we have $\nu_{ik}(0,0) = \nu/\chi_2$. The parameter χ_1 varies within the interval $1 \le \chi_1 \le \chi_2$. When $\chi_1 = 1$, binding of a regulatory molecule to the opposite subunit has no effect on the rate of binding by a given subunit, and thus cooperativity is absent. On the other hand, when $\chi_1 = \chi_2$, only binding of regulatory molecules to both subunits increases the binding probability for the substrate, and hence cooperativity reaches its maximal efficiency. This means that the parameter χ_1 can be taken here to specify the degree of cooperativity inside the molecule.

If the enzyme is *inhibited* by the binding of regulatory product molecules in the sequential model, the probability rate ν_{ij} of binding a substrate molecule is $\nu_{ij}(1,1) = \nu/\chi_2$ if both regulatory sites are occupied. If only the regulatory site of a given subunit is occupied and the other site is vacant, the binding rate for the respective subunit is $\nu_{ik}(1,0) = \nu/\chi_1$. If the regulatory site of the subunit is vacant, but the regulatory site of the other subunit is occupied, then $\nu_{ik}(0,1) = \nu$. Finally, if both regulatory sites are vacant, we have $\nu_{ik}(0,0) = \nu$. The parameter χ_1 again varies in the interval $1 \le \chi_1 \le \chi_2$.

In enzymes with heterotropic cooperativity, the binding probability rate β of regulatory molecules and their dissociation rate κ are not influenced by interactions between the subunits. We assume in this paper that regulatory molecules can bind to a subunit k of enzyme i only when this subunit is in the ground state, i.e., when $s_{ik} = 0$.

(b) Homotropic Cooperativity. For the sequential mechanism of homotropic "positive" cooperativity, the probability rate β_{ik} of binding a regulatory molecule is $\beta_{ik}(0,0) = \beta/\chi_3$ if both binding sites are vacant. If one binding site is occupied, the probability rate for binding a regulatory molecule on the other subunit increases to $\beta_{ik}(0,1) = \beta$. For "negative" cooperativity, the probability rates to bind a regulatory molecule are $\beta_{ik}(0,0) = \beta$ and $\beta_{ik}(0,1) = \beta/\chi_3$, respectively. The parameter $\chi_3 > 1$ specifies the strength of cooperativity. Binding of a regulatory molecule switches the respective subunits to the active state in the case of *activation*. If no regulatory molecule is bound at the subunit under consideration, the substrate binding rate will be $\nu_{ik} = \nu/\chi_1$. Binding of a regulatory molecule will increase the substrate binding rate to $\nu_{ik} = \nu$. In the case of *inhibition*, the subunit enters the inactive state when it binds a regulatory molecule. Hence, the substrate binding rate decreases from $\nu_{ik} = \nu$ if no regulatory molecule is bound, to $\nu_{ik} = \nu/\chi_1$ after having bound a regulatory molecule. Binding of regulatory molecules to a subunit k of enzyme i can occur only when this subunit is in the ground state, i.e., if $s_{ik} = 0$. The dissociation rate κ of regulatory molecules is not influenced by intermolecular interactions.

We consider in this paper a simple enzymatic reaction according to the scheme

$$S + E \xrightarrow{\nu} ES \rightarrow E + P, \qquad P \xrightarrow{\gamma} 0 \tag{6}$$

where a substrate molecule S is first bound to an enzyme molecule at rate ν and then transformed and released as a product molecule P. We restrict our model to irreversible reactions. The substrate concentration is large and is not influenced by the reaction. The product decays or is converted by other chemical reactions at rate γ. A product molecule of this reaction can bind to an enzyme and regulate its activity, acting either as an allosteric activator or inhibitor.

The reaction volume is assumed to be so small that the conditions of a molecular network are satisfied.[22] Under these conditions, all diffusional transport times of regulatory molecules inside the reaction volume are much shorter than the duration of a single turnover cycle in an enzyme molecule. Hence, a regulatory molecule released by a given enzyme can influence with equal probability the catalytic cycle of any other enzyme molecule in the population and the system behavior does not depend on the coordinates of involved molecules. The reaction is thus modeled as a collective dynamical process in a population of molecular machines (individual enzyme molecules) that communicate by releasing regulatory product molecules.

Several processes can change the number m of free product molecules. Binding to or dissociation from a subunit decreases or increases the number of free product molecules. A product molecule is formed when a subunit reaches the point $\phi_{ik} = \phi_p$ on the reaction coordinate. Removal of product molecules occurs by decay at probability rate γ, modeling the further processing of product molecules downstream a reaction pathway by, e.g., other enzymatic reactions. We assume that the mean lifetime of free product molecules is much shorter than the duration of an enzymic turnover cycle, so that $\gamma^{-1} \ll \tau$. The detailed algorithms used in numerical simulations of this stochastic model are presented in the Appendix.

2. Intermolecular vs Intramolecular Synchronization of Turnover Cycles

In this section we present the results of numeric simulations of the stochastic model. In our previous publications[25,27,28] we have defined a special order parameter that measures the degree of synchronization of enzymic turnover cycles. A similar parameter can be used to quantify the degree of *intermolecular synchronization* of turnover cycles in any two functional subunits belonging to different enzyme molecules. The statistical description of mutual synchronization is provided by the distribution over phase differences $P(\Delta\phi)$, specifying the probability density to find a phase difference $\Delta\phi$ between any two functional units belonging to different enzymes:

$$P(\Delta\phi) = \langle [\sum_{\substack{i,j=1 \\ i\neq j}}^{N} \sum_{k,l=1}^{2} s_{ik}s_{jl}]^{-1} \sum_{\substack{i,j=1 \\ i\neq j}}^{N} \sum_{k,l=1}^{2} s_{ik}s_{jl}\delta(\phi_{ik} - \phi_{jl} - \Delta\phi)\rangle_t \tag{7}$$

In this equation, $\delta(x)$ is the delta-function, and s_{ik} is the earlier defined binary variable, which is $s_{ik} = 0$ if the subunit k of enzyme i is in the free state, and $s_{ik} = 1$ if this subunit is within

its reaction cycle; note that thus the summation is performed effectively only over enzyme subunits inside their cycles. The distribution is normalized by the factor in the square brackets; the expression inside the angular brackets is averaged over time to eliminate fluctuations. Using the distribution over phase differences, the order parameter θ, which characterizes the strength of intermolecular synchronization of the turnover cycles of subunits belonging to *different* enzymes in the whole population, can be defined as

$$\theta = \int_{-0.5}^{0.5} (P(\Delta\phi) - 1)^2 \, d\Delta\phi \tag{8}$$

If intermolecular synchronization is absent in the system, then $\theta = 0$. Higher values of θ indicate a higher degree of synchronization of turnover cycles of different enzymes.

Further, we introduce another synchronization parameter θ_s that measures the degree of *intramolecular synchronization* of turnover cycles between two subunits belonging to *the same* enzyme molecule. First, we define distribution over phase differences $P_s(\Delta\phi)$ between two subunits within the same enzyme molecule:

$$P_s(\Delta\phi) = \langle [\sum_{i=1}^{N} \sum_{\substack{k,l=1 \\ k \neq l}}^{2} s_{ik} s_{il}]^{-1} \sum_{i=1}^{N} \sum_{\substack{k,l=1 \\ k \neq l}}^{2} s_{ik} s_{il} \delta(\phi_{ik} - \phi_{il} - \Delta\phi) \rangle_t \tag{9}$$

Here the index i refers to the enzyme while the second index specifies a subunit in the respective enzyme. The intramolecular synchronization order parameter θ_s is defined as

$$\theta_s = \int_{-0.5}^{0.5} (P_s(\Delta\phi) - 1)^2 \, d\Delta\phi \tag{10}$$

We have performed a series of numerical simulations of the enzymic reaction (eq 1) in the regime of a molecular network, using the algorithm specified in the Appendix. The total number of enzymes was always $N = 200$, and each enzyme consisted of two subunits. We always had $\chi_2 = 10^4$, $\upsilon = 1$, and $\tau = 1/\upsilon = 1$. At the beginning of the simulation, all enzyme subunits were randomly distributed over their cycle phases. The simulations were performed, using various assumptions about the mechanisms of intramolecular cooperativity, both in the case of activation and inhibition.The simulation data were statistically processed to determine the synchronization parameters.

(a) Heterotropic Cooperativity. Figure 2a shows the computed dependence of the intermolecular (θ) and intramolecular (θ_s) synchronization parameters on the strength of heterotropic cooperativity χ_1 for the case of *product activation*. If cooperativity is absent ($\chi_1 = 1$), both synchronization parameters coincide. This means that, as may be expected, the synchronization of two subunits does not depend on whether they belong to the same enzyme or not. This regime persists if cooperativity is present but remains relatively weak.

An increase of cooperativity χ_1 leads to an interesting effect: Both synchronization parameters diminish, reaching very small values. Hence, both synchronization inside an enzyme molecule and between different molecules disappear. When cooperativity χ_1 is further increased, synchronization reappears. However, in this new regime intramolecular synchronization is much stronger than intermolecular synchronization between subunits belonging to different enzyme molecules.

To clarify the origin of this complex synchronization behavior, we additionally display in Figure 3 for two cooperativities χ_1 the computed probability distributions $P(\Delta\phi)$ and $P_s(\Delta\phi)$ over phase differences between subunits belonging to different or to the same enzymes. The synchronous regimes found in this system at low cooperativity (Figure 3a) are characterized by the presence of two coherent groups of enzyme subunits that operate with a phase shift of half a period (i.e., the probability

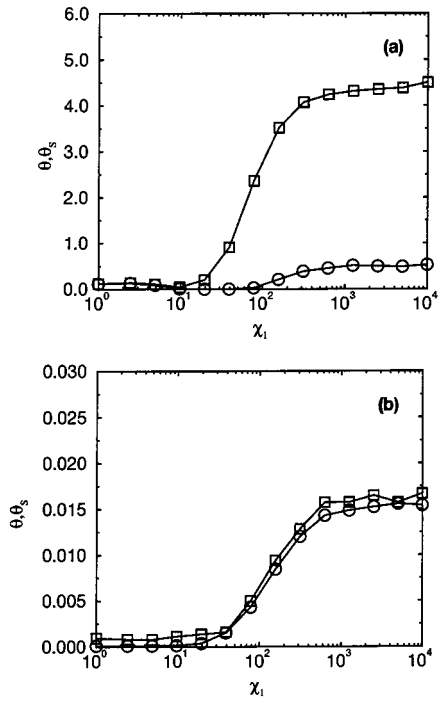

Figure 2. Order parameters θ (circles) and θ_s (squares) as functions of the strength of cooperativity: (a) heterotropic cooperativity for product-activated enzymatic reaction; (b) heterotropic cooperativity for product-inhibited enzymatic reaction. Reaction parameters: (a) $S = 1000$, $\alpha = 0.4$, $\nu = 400$, $\beta = 2$, $\kappa = 20$, $\gamma = 25$, $\xi = 0.06$, $\tau = 1$; (b) $\nu = 100$, $\beta = 5$, $\kappa = 30$, $\gamma = 15$, $\xi = 0.06$, $\tau = 1$.

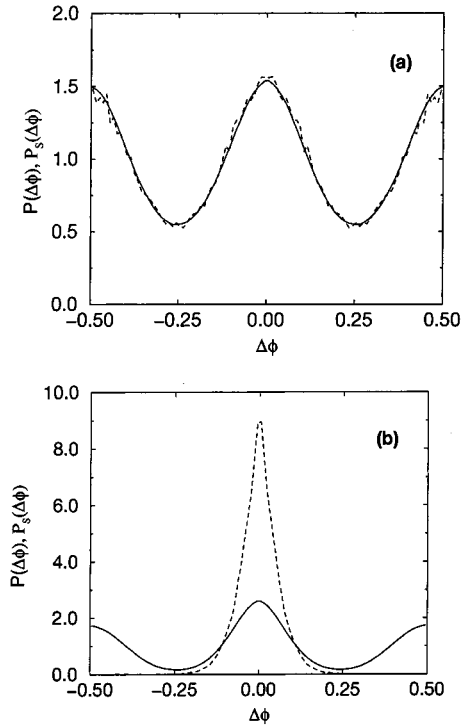

Figure 3. Distribution over phase differences for (a) $\chi_1 = 2.44$, and (b) $\chi_1 = 1250$. The solid line represents the distribution $P(\Delta\phi)$ over phase cycles among the total enzyme population; the dotted line indicates distribution $P_s(\Delta\phi)$ over phase cycles between the subunits within enzymes. Other reaction parameters are as in Figure 2a.

distributions have two maxima at $\Delta\phi = 0$ and $\Delta\phi = 0.5$). In this case, both probability distributions coincide and, therefore, the same synchronization behavior is found for subunits belonging to the same or to different molecules. In contrast to this, when cooperativity is high (Figure 3b), the synchronization behavior of subunits is different. Subunits belonging to different enzymes still tend to form two coherent groups shifted by half a period. However, the subunits inside the same molecules tend to synchronize their cycles and therefore the intramolecular probability distribution $P_s(\Delta\phi)$ shows only one maximum located at $\Delta\phi = 0$.

Hence, intramolecular interactions are acting in this case *against* allosteric interactions in the system and tend to impose different kinds of synchronization. When they are weak, allosteric effects dominate. When cooperativity is strong, it imposes perfect synchronization of cycles of subunits inside a molecule, while the cycles of different molecules may still be shifted. A transition from one synchronization mode to another takes place at intermediate cooperativity strengths and is accompanied by the suppression of both intramolecular and intermolecular synchronizations.We see that, if cooperative intramolecular interactions are present, one should distinguish between the coherent dynamics of subunits in the same molecule and the coherent dynamics of the entire enzyme population. These intramolecular interactions can interfere with allosteric self-regulation of the reaction so that their interplay determines the developing collective dynamical regime of this molecular network.

In our model of enzymic reaction cycles, a diffusive component is superimposed on the drift motion along the one-dimensional reaction coordinate (see eq 2). This diffusive component is realized by addition of white Gaussian noise of intensity σ. Noise leads to statistical variation of durations of turnover cycles of individual subunits, which is characterized by dispersion ξ. The synchronization regimes displayed in Figure 2a correspond to the statistical dispersion of molecular turnover times of 6% ($\xi = 0.06$). Intermolecular synchronization can be observed up to the relatively high statistical dispersions of 20% (cf. ref 27). We have found that, even when intermolecular synchronization is destroyed by fluctuations, synchronization of cycles in two subunits inside the same molecules can still be present at sufficiently high cooperativities. As an example, Figure 4a shows the dependence of the two synchronization parameters on the cooperativity χ_1 at the statistical dispersion of turnover times reaching 40%. Figure 4b displays the probability distributions for phase differences between subunits inside the same molecule and for different molecules at the cooperativity $\chi_1 = 1250$ for the same level of intramolecular noise.

As previously demonstrated,[25] allosteric product activation can lead to the synchronization characterized by different numbers of coherent enzymic groups. However, the regime with two coherent groups, shifted by half the cycle period, is the most robust with respect to the action of intramolecular noise. Therefore, only this synchronization regime has been chosen here to study the effects of intramolecular cooperativity.

The role of heterotropic cooperativity has also been investigated by us for allosteric *product inhibition*. Our previous analysis[27] has shown that in this case, in the absence of cooperativity, the enzymes always undergo synchronization into a single coherent group if allosteric regulation is sufficiently strong. This means that intramolecular interactions, tending to synchronize the cycles of subunits in the same molecules, will never act here against allosteric intermolecular interactions.

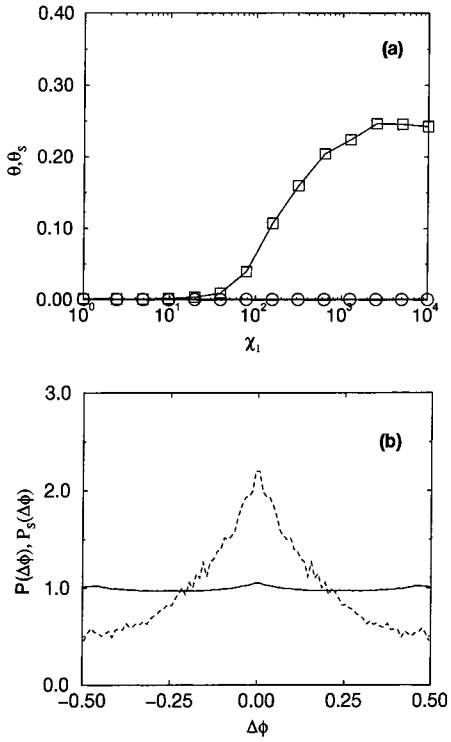

Figure 4. (a) Order parameters θ (circles) and θ_s (squares) as functions of the strength of cooperativity for the product-activated enzymatic reaction with heterotropic cooperativity at a statistical dispersion of turnover times $\xi = 0.4$. (b) Distribution over phase differences for $\chi_1 = 1250$ at a statistical dispersion of turnover times $\xi = 0.4$. The solid line represents the distribution $P(\Delta\phi)$ over phase cycles among the total enzyme population; the dotted line indicates distribution $P_s(\Delta\phi)$ over phase cycles between the subunits within enzymes. Other reaction parameters are as in Figure 2a.

Indeed, our simulations have shown that the two synchronization parameters θ and θ_s always almost coincide in the system with allosteric inhibition (Figure 2b).

(b) Homotropic Cooperativity. Figure 5a shows the dependence of the synchronization parameters θ and θ_s on the cooperativity parameter χ_3 for the homotropic *positive* cooperativity of a *product-activated* enzymatic reaction. As in the case of heterotropic cooperativity for the product-activated enzymatic reaction, there are two coherently acting groups of enzymes here. Homotropic positive cooperativity is found to have a strong effect on intermolecular synchronization. The corresponding synchronization parameter θ increases with χ_3 to reach a maximum, and then decreases to zero. In contrast to this, the intramolecular synchronization parameter θ_s grows with χ_3 and remains high when intermolecular synchronization of turnover cycles has nearly disappeared.

The respective case of homotropic positive cooperativity for the *product-inhibited* enzymatic reaction is shown in Figure 5b. There is only one coherent group of enzymes present in this system. Now, both synchronization parameters θ and θ_s decrease rapidly when cooperativity parameter χ_3 increases. Hence, strong cooperativity destroys in this case not only the intermolecular, but also the intramolecular synchronization.

Finally, Figure 6 shows the dependence of synchronization parameters θ and θ_s on the cooperativity parameter χ_3 for the *product-activated* (Figure 6a) and *product-inhibited* (Figure 6b) enzymatic reaction with homotropic *negative* cooperativity. Both synchronization parameters coincide now. Remarkably, the synchronization is found in such systems only in an interval of the cooperativity parameter.

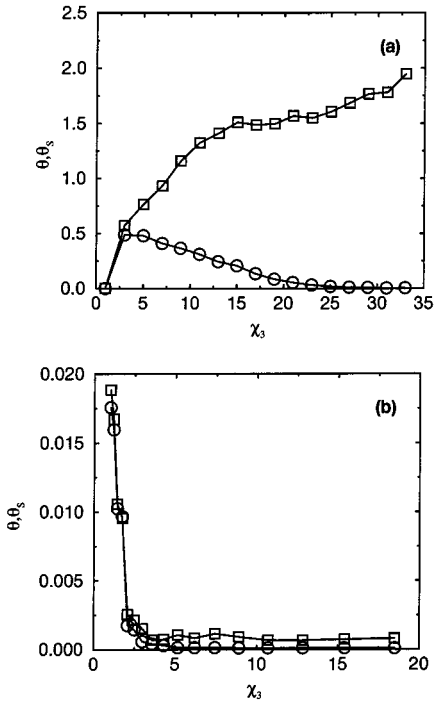

Figure 5. Order parameters θ (circles) and θ_s (squares) as functions of the strength of cooperativity: (a) homotropic positive cooperativity for product-activated enzymatic reaction; (b) homotropic positive cooperativity for product-inhibited enzymatic reaction. Reaction parameters: (a) $\nu = 200$, $\beta = 8$, $\kappa = 30$, $\gamma = 30$, $\xi = 0.06$; (b) $\nu = 100$, $\beta = 20$, $\kappa = 30$, $\gamma = 30$, $\xi = 0.06$.

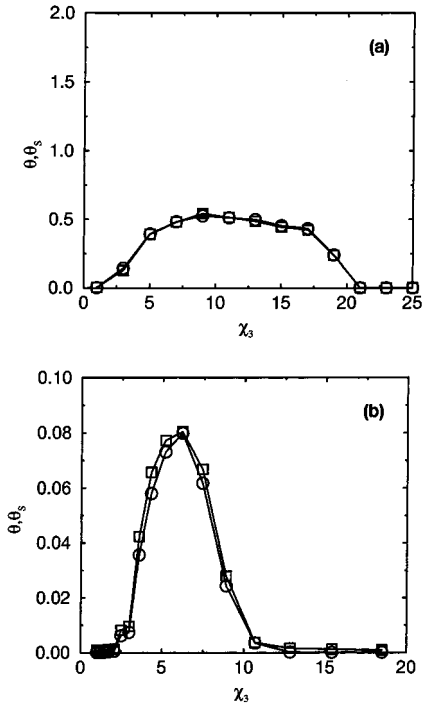

Figure 6. Order parameters θ (circles) and θ_s (squares) as functions of the strength of cooperativity: (a) homotropic negative cooperativity for product-activated enzymatic reaction; (b) homotropic negative cooperativity for product-inhibited enzymatic reaction. Reaction parameters: (a) $\nu = 200$, $\beta = 0.3$, $\kappa = 60$, $\gamma = 40$, $\xi = 0.06$; (b) $\nu = 200$, $\beta = 4$, $\kappa = 30$, $\gamma = 20$, $\xi = 0.06$.

3. Mean-Field Approximation

The mean-field approximation for the considered systems is obtained if we neglect stochastic fluctuations of reactants in the limit $N \rightarrow \infty$. Previously, we have discussed the synchronization behavior in this approximation for enzymes with a single functional unit (see refs 25, 27, and 28). Below in this section the equations of the mean-field approximation are derived for the enzymes with two functional subunits and cooperative interactions between them. Only the case of heterotropic cooperativity and the product-activated reaction will be considered here.

We introduce three density functions, $\tilde{n}_0(\phi,t)$, $\tilde{n}_1(\phi,t)$, and $\tilde{n}_2(\phi,t)$, that specify distributions over phases ϕ at time t of enzyme subunits belonging to enzymes having no, one, or two bound regulatory molecules, respectively. Thus, for example, $\tilde{n}_0(\phi,t)\Delta\phi$ is the mean number of enzyme subunits that have not bound a regulatory molecule and have phases in the interval from ϕ to $\phi + \Delta\phi$ at time t. Additionally, mean numbers $n_0(t)$, $n_1(t)$, and $n_2(t)$ of subunits in the ground state belonging to enzymes, having bound no, one, or two regulatory molecules, should be specified. The mean number of free product molecules is $m(t)$.

The mean-field evolution equations of our system for $\tilde{n}_0(\phi,t)$, $\tilde{n}_1(\phi,t)$, and $\tilde{n}_2(\phi,t)$ are (cf. refs 25, 27, and 28)

$$\frac{\partial \tilde{n}_0(\phi,t)}{\partial t} = -\upsilon\frac{\partial \tilde{n}_0(\phi,t)}{\partial \phi} + \kappa\tilde{n}_1(\phi,t) + \sigma\frac{\partial^2 \tilde{n}_0(\phi,t)}{\partial \phi^2} \quad (11)$$

$$\frac{\partial \tilde{n}_1(\phi,t)}{\partial t} = -\upsilon\frac{\partial \tilde{n}_1(\phi,t)}{\partial \phi} + \kappa\tilde{n}_1(\phi,t) + 2\kappa\tilde{n}_2(\phi,t) + \sigma\frac{\partial^2 \tilde{n}_1(\phi,t)}{\partial \phi^2} \quad (12)$$

$$\frac{\partial \tilde{n}_2(\phi,t)}{\partial t} = -\upsilon\frac{\partial \tilde{n}_2(\phi,t)}{\partial \phi} + \kappa\tilde{n}_2(\phi,t) + \sigma\frac{\partial^2 \tilde{n}_2(\phi,t)}{\partial \phi^2} \quad (13)$$

The right-hand side of the evolution equations consists of terms describing the drift motion at constant velocity υ and superimposed diffusion with intensity σ along the reaction coordinate ϕ. Further, there are terms that take into account the dissociation of bound regulatory molecules at rate κ. There are no terms for the binding of regulatory molecules because in our model regulatory molecules can bind only to enzyme subunits in the ground state, whereas the evolution equations describe the distribution of enzyme subunits within their reaction cycle. To simplify our calculations, we shall later assume that intramolecular fluctuations are absent ($\sigma = 0$).

We have the following boundary conditions for the evolution eqs 11− 13 for the considered sequential model of heterotropic cooperativity:

$$\tilde{n}_0(\phi,t)|_{\phi=0} = \frac{\nu_0}{\upsilon}n_0(t) \quad (14)$$

$$\tilde{n}_1(\phi,t)|_{\phi=0} = \frac{\nu_0 + \nu_1}{2\upsilon}n_1(t) \quad (15)$$

$$\tilde{n}_2(\phi,t)|_{\phi=0} = \frac{\nu_2}{\upsilon}n_2(t) \quad (16)$$

Here $\nu_0 = \nu/\chi_2$ is the binding rate of substrate molecules to a subunit that has not bound a regulatory molecule. For a subunit belonging to enzymes that have bound one regulatory molecule, substrate molecules bind at rate $\nu_1 = \nu/\chi_1$ to the subunit with the actually occupied regulatory binding site and at rate $\nu_0 = \nu/\chi_2$ to the other subunit with the empty regulatory binding site. Substrate molecules bind at rate $\nu_2 = \nu$ to subunits belonging to enzymes that have bound regulatory molecules to both of

their subunits. The rate $\nu = \alpha S$ is proportional to the number S of substrate molecules in the system. The boundary conditions express that the number of subunits entering the reaction cycle is proportional to the number of subunits in the ground state and the respective substrate binding rate.

Further, there are evolution equations for the mean number of enzyme subunits in the ground state, which have bound no, one, or two regulatory molecules:

$$\frac{dn_0}{dt} = -2\beta m(t)\, n_0(t) + \kappa n_1(t) - \nu_0 n_0(t) + \upsilon \tilde{n}_0(1,t) \quad (17)$$

$$\frac{dn_1}{dt} = 2\beta m(t)\, n_0(t) - \beta m(t)\, n_1(t) - \kappa n_1(t) + 2\kappa n_2(t) - \frac{\nu_0 + \nu_1}{2} n_1(t) + \upsilon \tilde{n}_1(1,t) \quad (18)$$

$$\frac{dn_2}{dt} = \beta m(t)\, n_1(t) + 2\kappa n_2(t) - \nu_2 n_2(t) + \upsilon \tilde{n}_2(1,t) \quad (19)$$

The terms on the right-hand side of these equations take into account that free product molecules can bind as regulatory molecules at rate β to enzyme subunits in the ground state if there are binding sites available. Note that the overall rate of product binding to enzyme subunits belonging to enzymes that have bound no regulatory molecules is twice as high as the overall binding rate to enzyme subunits belonging to enzymes that have already bound a regulatory molecule to one of their subunits. As in the evolution equations for the enzyme subunits within their reaction cycles, here we also have terms that describe the dissociation of bound regulatory molecules at rate κ. Further, depending on the number of bound regulatory molecules, enzyme subunits can bind substrate molecules at rates ν_0, ν_1, or ν_2, and enter their reaction cycle. These binding rates describe the binding to individual subunits; thus, for the sequential model of heterotropic cooperativity, the overall binding rate to subunits belonging to an enzyme having bound one regulatory molecule is $(\nu_0 + \nu_1)/2$. Finally, the number of enzyme subunits in the ground state can be increased by subunits having completed their reaction cycle. This is expressed by the last term in the evolution equations for the enzyme subunits in the ground state.

We also have an evolution equation for the number of free product molecules

$$\frac{dm}{dt} = -\gamma m(t) + \upsilon \tilde{n}_0(\phi_p,t) + \upsilon \tilde{n}_1(\phi_p,t) + \upsilon \tilde{n}_2(\phi_p,t) + \frac{1}{2}\kappa n_1(t) + \kappa n_2(t) - \beta m(t)\, n_0(t) - \frac{1}{2}\beta m(t)\, n_1(t) + \frac{1}{2}\kappa \int_0^\tau \tilde{n}_1(\phi,t)\, d\phi + \kappa \int_0^\tau \tilde{n}_2(\phi,t)\, d\phi \quad (20)$$

The first term on the right-hand side of this equation describes the decay of free product molecules at rate γ. The next three terms correspond to the release of product molecules at the phase point $\phi = \phi_p$ of the reaction cycle of enzyme subunits. Further, we have terms for the dissociation of bound regulatory product molecules from subunits in the ground state, leading to an increase of the number of free product molecules, as well as terms for the binding of free product molecules to the regulatory site of enzyme subunits in the ground state, which leads to a decrease of free product molecules. The last two terms take into account the dissociation of regulatory product molecules from subunits within their reaction cycle.

The total number N of all enzyme subunits is obviously conserved and is given by

$$N = n_0(t) + n_1(t) + n_2(t) + \int_0^\tau \tilde{n}_0(\phi,t)\, d\phi + \int_0^\tau \tilde{n}_1(\phi,t)\, d\phi + \int_0^\tau \tilde{n}_2(\phi,t)\, d\phi \quad (21)$$

i.e., by a sum of enzyme subunits in the ground state and of enzyme subunits within their reaction cycles.

Equations 11−20 represent the mean-field approximation of our system. For $\sigma = 0$ eqs 11−13 can be solved to give

$$\tilde{n}_0(\phi,t) = \frac{\nu_0}{\upsilon} n_0\left(t - \frac{\phi}{\upsilon}\right) + \frac{\nu_0 + \nu_1}{2\upsilon}\left(1 - \exp\left(-\frac{\kappa}{\upsilon}\phi\right)\right) n_1\left(t - \frac{\phi}{\upsilon}\right) + \frac{\nu_2}{\upsilon}\left(1 - 2\exp\left(-\frac{\kappa}{\upsilon}\phi\right) + \exp\left(-2\frac{\kappa}{\upsilon}\phi\right)\right) n_2\left(t - \frac{\phi}{\upsilon}\right) \quad (22)$$

$$\tilde{n}_1(\phi,t) = \frac{\nu_0 + \nu_1}{2\upsilon} \exp\left(-\frac{\kappa}{\upsilon}\phi\right) n_1\left(t - \frac{\phi}{\upsilon}\right) + \frac{2\nu_2}{\upsilon} \exp\left(-\frac{\kappa}{\upsilon}\phi\right)\left(1 - \exp\left(-\frac{\kappa}{\upsilon}\phi\right)\right) n_2\left(t - \frac{\phi}{\upsilon}\right) \quad (23)$$

$$\tilde{n}_2(\phi,t) = \frac{\nu_2}{\upsilon} \exp\left(-2\frac{\kappa}{\upsilon}\phi\right) n_2\left(t - \frac{\phi}{\upsilon}\right) \quad (24)$$

Now we substitute these solutions (eqs 22−24) into eqs 17−20, using the definitions $\tau = 1/\upsilon$, $\tau_1 = \phi_p/\upsilon$ and setting $\upsilon = 1$ so that we get the final set of evolution equations

$$\frac{dn_0}{dt} = -2\beta m(t)\, n_0(t) + \kappa n_1(t) - \nu_0 n_0(t) + \nu_0 n_0(t-\tau) + \frac{\nu_0 + \nu_1}{2}(1 - \exp(-\kappa\tau)) n_1(t-\tau) + \nu_2(1 - 2\exp(-\kappa\tau) + \exp(-2\kappa\tau)) n_2(t-\tau) \quad (25)$$

$$\frac{dn_1}{dt} = 2\beta m(t)\, n_0(t) - \beta m(t)\, n_1(t) - \kappa n_1(t) + 2\kappa n_2(t) - \frac{\nu_0 + \nu_1}{2} n_1(t) + \frac{\nu_0 + \nu_1}{2} \exp(-\kappa\tau) n_1(t-\tau) + 2\nu_2 \exp(-\kappa\tau) n_2(t-\tau) - 2\nu_2 \exp(-2\kappa\tau) n_2(t-\tau) \quad (26)$$

$$\frac{dn_2}{dt} = \beta m(t)\, n_1(t) - 2\kappa n_2(t) - \nu_2 n_2(t) + \nu_2 \exp(-2\kappa\tau) n_2(t-\tau) \quad (27)$$

$$\frac{dm}{dt} = -\gamma m(t) + \nu_0 n_0(t-\tau_1) + \frac{\nu_0 + \nu_1}{2} n_1(t-\tau_1) + \nu_2 n_2(t-\tau_1) + \frac{1}{2}\kappa n_1(t) + \kappa n_2(t) - \beta m(t)\, n_0(t) - \frac{1}{2}\beta m(t)\, n_1(t) + \frac{\nu_0 + \nu_1}{4} \int_0^\tau \exp(-\kappa t') n_1(t-t')\, dt' + \nu_2\kappa \int_0^\tau \exp(-\kappa t') n_2(t-t')\, dt' \quad (28)$$

Mean-field eqs 25−28 describe the classical asynchronous regime of the considered enzymatic reaction and a transition to the coherent regime with periodic spiking of the product concentration.

Since fluctuations are neglected in the mean-field approximation, the asynchronous steady state corresponds to a fixed point of these equations. Assuming that n_0, n_1, n_2, and m are constant, a set of algebraic equations for these variables is derived. Figure

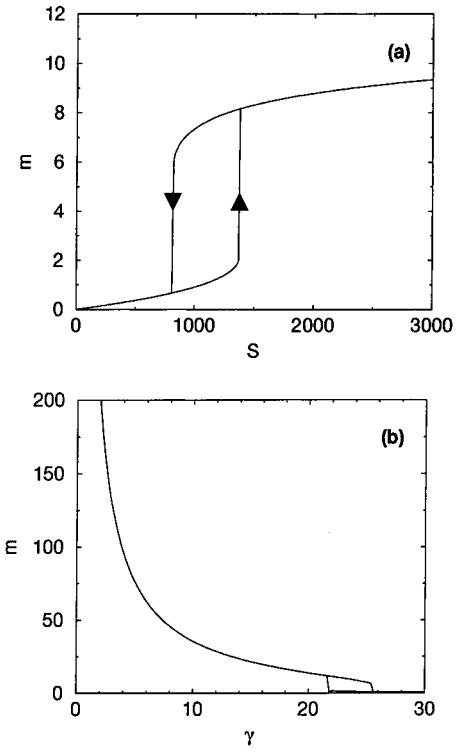

Figure 7. Number *m* of product molecules (a) as a function of the number of substrate molecules *S* in the system, calculated numerically from the fixed point of the mean-field eqs 25−28 for $\gamma = 25$ and (b) as a function of the decay rate γ of product molecules, calculated numerically from the fixed point of the meanfield eqs 25−28 for $S = 1000$. Other parameters are $\alpha = 0.4$, $\chi_1 = 2500$, $\beta = 0.5$, $\kappa = 20$, $\tau = 1$.

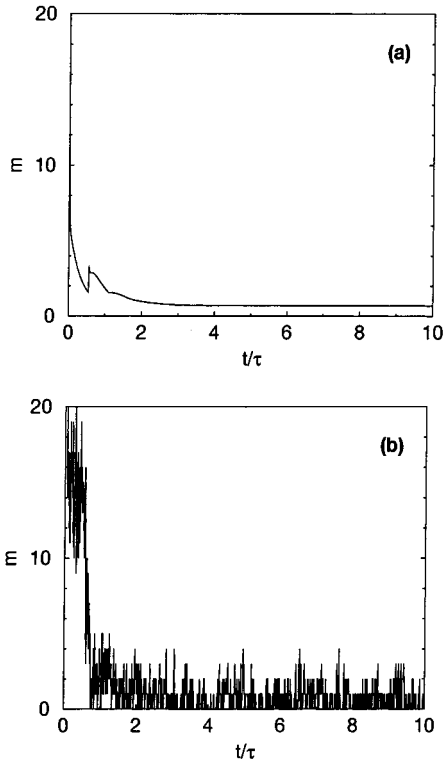

Figure 8. Time dependence of the number *m* of product molecules for the numerically integrated mean-field equations (a) and the stochastic model (b) for $\chi_1 = 1250$, $\beta = 0.3$, and $\xi = 0$. Other reaction parameters are as in Figure 2a.

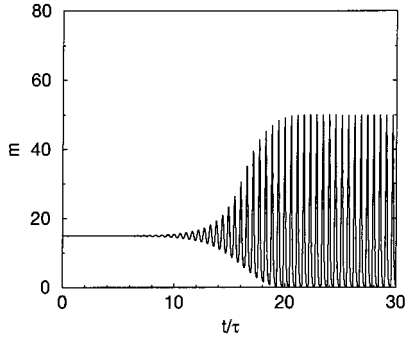

Figure 9. Development of oscillations in the number *m* of free product molecules starting from the fixed point for $\chi_1 = 1000$. Other reaction parameters as in Figure 2a.

7a shows the numerically computed dependence of the number *m* of free product molecules in this steady state as function of the number *S* of substrate molecules in the system (we have taken $\nu = \alpha S$ here). This is an S-shaped curve typical of enzymes with the cooperative behavior. For high substrate concentrations the curve becomes horizontal, since saturation takes place. It is interesting that, in an interval of substrate concentrations *S*, bistability is observed. Depending on the initial conditions, the system can be found either in a highly reactive state, characterized by a large product concentration, or in a state with low reactivity. Figure 7b shows how the number *m* of free product molecules depends on the product decay rate γ.

Figure 8a displays the results of a numerical integration of full time-dependent mean-field eqs 25−28 for the parameter values, yielding a stable asynchronous regime. After a transient the number *m* of free product molecules approaches a stationary level corresponding to the stable fixed point. For comparison, Figure 8b shows the respective simulation of the stochastic model, starting with a similar initial condition. As should be expected, the stochastic model reveals statistical fluctuations in the number of free product molecules in the system. The fluctuations become Poissonian in the steady state.

However, the steady state of the system, corresponding to a stationary solution of mean-field eqs 25−28, can also be unstable. When this occurs, rapid periodic oscillations in the number of free product molecules develop. Figure 9 displays the development of such oscillations.

Figure 10 shows the spiking regimes for the same parameter values in the mean-field approximation (Figure 10a) and in the full stochastic model (Figure 10b). The mean amplitude and period of the spiking are the same, although statistical fluctuations are absent in the mean-field approximation. Thus, when the analysis of statistical fluctuations is not needed, the mean-field equations can be used as a satisfactory approximation to describe both asynchronous and coherent regimes of the considered enzymic reaction in small spatial volumes.

4. Discussion

Previously, we have shown that enzymic reaction kinetics in small spatial volumes of micrometer sizes has special properties. A population of allosteric enzymes may then form a molecular network where individual molecular machines are communicating via small regulatory molecules. As a result, strong correlations between turnover cycles of single enzyme molecules can develop. This leads either to the synchronization of intramolecular dynamics in the entire enzymic population or to the formation of two or more coherent molecular groups. Inside a group, the turnover cycles of enzymes are synchronous. The cycles of enzymes belonging to different groups are shifted in phase by a fraction of a turnover period.

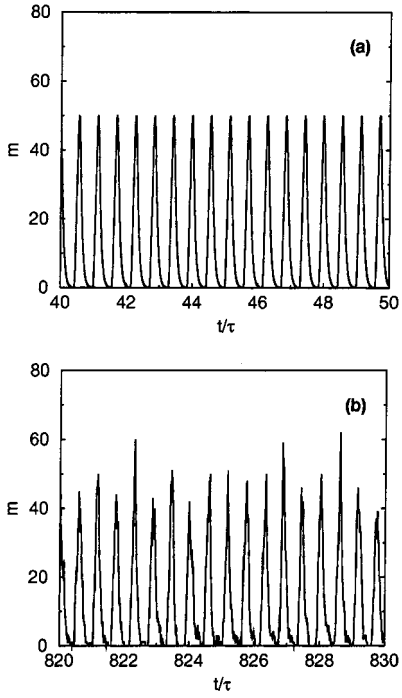

Figure 10. Time dependence of the number *m* of product molecules for the numerically integrated mean-field eqations (a) and the stochastic model (b) for $\chi_1 = 1250$ and $\xi = 0$. Other reaction parameters are as in Figure 2a.

In the present paper we have studied the synchronization phenomena in molecular networks formed by enzymes with several interacting subunits. Our study was based on a new stochastic model of an enzyme with two interacting subunits, representing an extension of the sequential model of Koshland et al.[31] Systems with heterotropic and homotropic (positive and negative) cooperativity were considered. Situations with allosteric product activation and product inhibition of enzymic cycles were separately analyzed.

We have shown that interactions between subunits in an enzyme molecule can significantly influence intermolecular mutual synchronization of turnover cycles, enhancing or weakening it. The latter effect is observed for product-activated reactions with heterotropic or homotropic positive cooperativity. In this case, allosteric interactions between molecules tend to form two coherently operating enzymic groups. If interactions between subunits in an enzyme molecule were absent, each of the subunits would have behaved as an independent enzyme molecule and, therefore, two subunits of the same molecule could belong to different enzymic groups and their turnover cycles could be anticorrelated. Interactions between subunits tend to synchronize their internal cycles and therefore act in this case against the effects of allosteric interactions. Generally, we have found that interactions between subunits in an enzyme molecule lead to the development of correlations between their turnover cycles. Such intramolecular synchronization can persist even at high fluctuation intensity, when statistical dispersion of individual turnover cycles reaches 40%.

Proceeding from the stochastic model, we have derived mean-field evolution equations for the collective dynamics of a molecular reaction network formed by allosteric product-activated enzymes with heterotropic cooperativity. This system of differential delay equations corresponds to an approximation where statistical fluctuations in the number of product molecules are neglected. Thus, the asynchronous steady state of the stochastic model with Poissonian fluctuations in the number of product molecules corresponds here to a stationary state with a constant product concentration. The synchronization of molecular turnover cycles, leading to rapid spiking in the number of products, is described in the mean-field approximation as the development of frequent periodic oscillations of the product concentration. By comparing the data of parallel simulations of the stochastic model and of the system of mean-field differential equations, we have shown that these equations correctly reproduce the mean amplitude and the period of product spikes, while neglecting their fluctuations.

Our analysis of mean-field equations for the case of heterotropic cooperativity with product activation has also revealed that, under certain conditions, the considered enzymic reaction may show bistability in the asynchronous regime. A systematic investigation of such cooperativity effects will be performed separately.

In our present study we have restricted the analysis to enzymes with only two subunits. The extension of the model to the case of more than two subunits is straightforward, and we do not expect any principally new phenomena. However, the model would be then much more complicated, because various geometric arrangements of the subunits, influencing interactions between them, should be considered.

Only the synchronization phenomena for enzymes with sequential cooperativity[31] have been investigated in this paper. Though it is not the subject of our present publication, we have also performed some preliminary numerical simulations based on the model of concerted cooperativity of Monod−Wyman−Changeaux[32] and have not found any significant difference with respect to the synchronization phenomena.

The majority of enzymes are composed of several subunits, and the phenomenon of cooperativity plays an important role in biochemistry of the cell. However, it should be emphasized that the results of our theoretical analysis are applicable only when certain conditions are satisfied. The synchronization of turnover cycles is possible only when the statistical variation of turnover times in single enzyme molecules is relatively low. This is generally expected when a turnover cycle of an enzyme involves passing through a sequence of many conformational substates and, thus, the processes of slow conformational relaxation are essentially involved in the enzyme activity. The information on the intensity of such intramolecular fluctuations can be obtained using the methods of single molecule spectroscopy. However, only a few such experiments with selected enzymes have been performed so far, and no detailed systematic data for various enzymes are available.

Low intensity of intramolecular fluctuations is expected for cytochrome P-450 enzymes where the reaction cycle has been monitored by time-resolved X-ray crystallography at atomic resolution.[33] This could indicate that the majority of enzyme molecules went synchronously through their reaction cycles and that there was only one dominant path down the energy landscape during the conformational relaxation. Remarkably, external synchronization of turnover cycles by the application of periodic light pulses has been observed[16−18] for the same enzymic system. The cytochrome P-450 enzymes are composed of several subunits, and interactions between them may significantly influence the synchronization phenomenon.

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Appendix

In this Appendix we give the details of the algorithms used in our numerical simulations of the stochastic model. First, we discretize the stochastic differential eq 2 for equal small time steps Δt:

$$\phi_{ik}(t+\Delta t) = \phi_{ik}(t) + v\Delta t + \varsigma_{ik}\sqrt{\sigma\Delta t} \tag{29}$$

where ς_{ik} are independent Gaussian numbers with

$$\langle \varsigma_{ik}(t)\, \varsigma_{jl}(t')\rangle = 2\delta_{ik,jl}\delta(t-\tau') \tag{30}$$

The phase point $\phi_{ik} = 0$ cannot be passed into the negative range. Should this happen, we replace $\phi_{ik}(t+\Delta t)$ by $\phi_{ik}(t+\Delta t) = v\Delta t$. Neither can the phase point $\phi_{ik} = \phi_p$ of the product release be passed in the negative direction, because we assume that the product cannot bind again back to the enzyme after it has been released. Should the phase given by eq 29 cross the phase point ϕ_p backward, we set therefore $\phi_{ik}(t+\Delta t) = \phi_p + v\Delta t$. If the enzyme subunit reaches a phase $\phi_{ik} > 1$, it is at the end of the reaction cycle and returns to the ground state $\phi_{ik}(t+\Delta t) = 0$.

For the *heterotropic* model of intramolecular cooperativity, the updating algorithm for the binary state variable r_{ik} is

$$r_{ik}(t+\Delta t) = \begin{cases} 1, \text{ if } r_{ik}(t) = 0, \text{ with probability } \mu_{ik}\Delta t \\ 0, \text{ if } r_{ik}(t) = 0, \text{ with probability } 1 - \mu_{ik}\Delta t \\ 0, \text{ if } r_{ik}(t) = 1, \text{ with probability } \kappa\Delta t \\ 1, \text{ if } r_{ik}(t) = 1, \text{ with probability } 1 - \kappa\Delta t \end{cases} \tag{31}$$

where the probability rate μ_{ik} is

$$\mu_{ik} = m(t)\, \beta(s_{ik}) \tag{32}$$

with $\beta(s_{ik})$ being the product binding rate constant for a subunit k of enzyme i in the state characterized by s_{ik}. If $s_{ik} = 0$, i.e., the subunit is in the ground state, then $\beta(s_{ik}) = \beta$, and zero otherwise. The binary variable s_{ik} is updated according to

$$s_{ik}(t+\Delta t) = \begin{cases} 1, \text{ if } s_{ik}(t) = 0, \text{ with probability } \nu_{ik}(r_{i1},r_{i2})\Delta t \\ 0, \text{ if } s_{ik}(t) = 0, \text{ with probability } 1 - \nu_{ik}(r_{i1},r_{i2})\Delta t \\ 0, \text{ if } s_{ik}(t) = 1, \text{ with probability } \phi_{ik}(t) = 1 \\ 1, \text{ if } s_{ik}(t) = 1, \text{ with probability } \phi_{ik}(t) < 1 \end{cases} \tag{33}$$

where the substrate binding rate $\nu_{ik}(r_{i1},r_{i2})$ is given for the sequential model with *product activation* for $\nu_{i1}(r_{i1},r_{i2})$ by

$$\nu_{i1}(r_{i1},r_{i2}) = \begin{cases} \nu/\chi_2, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 0 \\ \nu/\chi_1, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 0 \\ \nu/\chi_2, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 1 \\ \nu, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 1 \end{cases} \tag{34}$$

and for $\nu_{i2}(r_{i1},r_{i2})$

$$\nu_{i2}(r_{i1},r_{i2}) = \begin{cases} \nu/\chi_2, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 0 \\ \nu/\chi_2, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 0 \\ \nu/\chi_1, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 1 \\ \nu, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 1 \end{cases} \tag{35}$$

The substrate binding rate $\nu_{ik}(r_{i1},r_{i2})$ for the sequential model with *product inhibition* is, for $\nu_{i1}(r_{i1},r_{i2})$,

$$\nu_{i1}(r_{i1},r_{i2}) = \begin{cases} \nu, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 0 \\ \nu/\chi_1, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 0 \\ \nu, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 1 \\ \nu/\chi_2, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 1 \end{cases} \tag{36}$$

and. for $\nu_{i2}(r_{i1},r_{i2})$.

$$\nu_{i2}(r_{i1},r_{i2}) = \begin{cases} \nu, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 0 \\ \nu, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 0 \\ \nu/\chi_1, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 1 \\ \nu/\chi_2, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 1 \end{cases} \tag{37}$$

For the *homotropic* model of *positive cooperativity*, updating of binary variables is performed according to the following rules. For $r_{ik}(t+\Delta t)$ we have

$$r_{ik}(t+\Delta t) = \begin{cases} 1, \text{ if } r_{ik}(t) = 0, \text{ with probability } \mu_{ik}\Delta t \\ 0, \text{ if } r_{ik}(t) = 0, \text{ with probability } 1 - \mu_{ik}\Delta t \\ 0, \text{ if } r_{ik}(t) = 1, \text{ with probability } \kappa\Delta t \\ 1, \text{ if } r_{ik}(t) = 1, \text{ with probability } 1 - \kappa\Delta t \end{cases} \tag{38}$$

The binding rate μ_{ik} of regulatory product molecules is

$$\mu_{ik} = m(t)\, \beta_{ik}(s_{ik},r_{ik}) \tag{39}$$

The probability rate $\beta(s_{ik},r_{i1},r_{i2})$ of the binding of a regulatory molecule is zero if $s_{ik} = 1$, i.e., if the subunit k is within the reaction cycle. If this subunit is in the ground state ($s_{ik} = 0$), then we have

$$\beta_{i1}(0,r_{i1},r_{i2}) = \begin{cases} \beta/\chi_3, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 0 \\ \beta, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 1 \\ 0, \text{ if } r_{i1} = 1 \end{cases} \tag{40}$$

and for $\beta_{i1}(0,r_{i1},r_{i2})$

$$\beta_{i2}(0,r_{i1},r_{i2}) = \begin{cases} \beta/\chi_3, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 0 \\ \beta, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 0 \\ 0, \text{ if } r_{i2} = 1 \end{cases} \tag{41}$$

The updating algorithm for the binary state variable s_{ik} is

$$s_{ik}(t+\Delta t) = \begin{cases} 1, \text{ if } s_{ik}(t) = 0, \text{ with probability } \nu_{ik}(r_{i1},r_{i2})\Delta t \\ 0, \text{ if } s_{ik}(t) = 0, \text{ with probability } 1 - \nu_{ik}(r_{i1},r_{i2})\Delta t \\ 0, \text{ if } s_{ik}(t) = 1, \text{ with probability } \phi_{ik}(t) = 1 \\ 1, \text{ if } s_{ik}(t) = 1, \text{ with probability } \phi_{ik}(t) < 1 \end{cases} \tag{42}$$

where, in case of *activation*, the substrate binding rate is $\nu_{ik} = \nu/\chi_1$ if $r_{ik} = 0$, and $\nu_{ik} = \nu$ if $r_{ik} = 1$. For the *product-inhibited* enzymatic reaction we have $\nu_{ik} = \nu$ if $r_{ik} = 0$, and $\nu_{ik} = \nu/\chi_1$ if $r_{ik} = 1$.

For the *homotropic* model of *negative cooperativity* eqs 40 and 41 read as

$$\beta_{i1}(0,r_{i1},r_{i2}) = \begin{cases} \beta, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 0 \\ \beta/\chi_3, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 1 \\ 0, \text{ if } r_{i2} = 1 \end{cases} \tag{43}$$

and for $\beta_{i2}(s_{ik}=0,r_{i1},r_{i2})$

$$\beta_{i2}(0,\, r_{i1},\, r_{i2}) = \begin{cases} \beta, \text{ if } r_{i1} = 0 \text{ and } r_{i2} = 0 \\ \beta/\chi_3, \text{ if } r_{i1} = 1 \text{ and } r_{i2} = 0 \\ 0, \text{ if } r_{i2} = 1 \end{cases} \tag{44}$$

For both the heterotropic and homotropic model of cooperativity the rule for the updating of the number m of free product molecules is

$$m(t+\Delta t) = m(t) + \sum_{i=1}^{N}\sum_{k=1}^{2}\Theta(\phi_{ik}(t) - \phi_p)\,\Theta(\phi_p - \phi_{ik}(t-\Delta t)) - \sum_{l=1}^{m(t)}\zeta_l + \sum_{i=1}^{N}\sum_{k=1}^{2}(r_{ik}(t) - r_{ik}(t+\Delta t)) \quad (45)$$

The definition of the Heaviside step function is $\Theta(x) = 1$ if $x \geq 0$, and $\Theta(x) = 0$ if $x < 0$. The binary random number ζ_l takes values 1 and 0 with probability $\gamma\Delta t$, and $1 - \gamma\Delta t$. The second term of eq 45 describes the release of product molecules when a subunit passes the phase point $\phi_{ik} = \phi_p$, and therefore increases the number m of free product molecules by 1. The stochastic process of decay of product molecules is expressed by the third term because any of the product molecules can decay with probability $\gamma\Delta t$ within the short time intervall Δt. The last term of eq 45 takes into account the events of binding and release of product molecules to the regulatory site of the subunits.

The time step Δt must be small enough, so that the conditions $\mu_{ik}\Delta t \ll 1$, $\gamma\Delta t \ll 1$, $\kappa\Delta t \ll 1$, and $\nu_{ik}\Delta t \ll 1$ hold.

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