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Enzymatic reactions in small spatial volumes: comment on a model of Hess and Mikhailov

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Abstract

Recently Hess and Mikhailov pointed out that in small subcellular compartments diffusion is so fast that mixing is instantaneous on the time scale of many enzymatic reactions. This opens the possibility for synchronizing individual reaction events. To illustrate this fact they discuss as example an irreversible enzymatic reaction with allosteric product activation. Under appropriate conditions their model shows coherent spiking in the number of product molecules, caused by the strong correlation between reaction events. In this model only substrate binding is an indeterministic process, all other subsequent transitions between different enzyme states being deterministic, contrary to real processes. The purpose of the present paper was to investigate this interesting phenomenon by means of a more realistic modification of the original model, with only probabilistic transitions. In an attempt to obtain spiking, which was not observed under these conditions, the model was extended to make a clear distinction between allosteric high and low affinity substrate binding, in contrast to the original model using a product dependent mean binding probability. However no periodic signal was detectable in the indeterministic version of the Hess Mikhailov model or the extended version, either by means of direct visualization or on autocorrelation or Fourier analysis. Reasons why spiking is not observed in indeterministic enzyme models are discussed. © 1998 Elsevier Science B.V.

Keywords: Enzymes; Molecular chaos; Oscillations; Coherent spiking; Allosteric activation; Turnover time; Stochastic enzyme kinetics

1. Introduction

Recently Hess and Mikhailov [1–6] pointed out that the timing of chemical reactions in small volumes depends on two factors: (i) mixing time, t_{mix}, the time after which a newly introduced molecule is found everywhere in the volume with equal probability; this time depends on the diffusion constant, D_1 and the linear dimension of the compartment, L_1 and is of the order of $t_{mix} \approx L^2/D$. (ii) Traffic time, t_{tr}, the mean time for an encounter of the molecule with a single target particle (e.g. an enzyme molecule). For t_{tr} the relation $t_{tr} = V/(4\pi DR)$ follows from Smoluchowski's reaction rate theory [7], whereby V is the compartment volume and R is the target radius for spherical particles. If both particle and target are mobile the effective diffusion constant is given by $D = D_{particle} + D_{target}$.

If there are N target particles the mean encounter time is $t_{enc} = t_{tr}/N$. During the time t_{enc} a substantial number of originally co-localized molecules

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spreads over a distance of about $(Dt_{enc})^{1/2}$, giving a spatial correlation of chemical reactions within a correlation length of $1_C \approx (Dt_{tr}/N)^{1/2}$. Therefore in a small enough compartment, with mixing times smaller than the mean encounter time ($t_{mix}/t_{enc} < 1$), synchronization throughout the whole compartment is possible, because mixing is instantaneous on the time scale of possible chemical reactions. From this condition it follows that spatial correlations are possible only providing a critical number $N_C \approx L/R$ of target particles is not exceeded.

On the basis of these spatial correlations, Hess and Mikhailov argue that subcellular compartments should show uncommon behavior in reaction kinetics. The following seemingly promising model [4,6] was presented to illustrate their viewpoint: from characteristic values for the linear dimensions of cell compartments ($L = 100$ nm), target groups ($R = 1$ nm), and diffusion constants ($D = 10^{-6}$ cm^2 s^{-1} = 10^5 nm^2 ms^{-1}), a system of about $N_C \approx 100$ enzyme molecules can show correlated behavior. The following catalytic cycle is proposed for these enzyme molecules (see Fig. 1A, with all transition probabilities $p_i = 1$ for $i > 0$): initially an enzyme molecule is in a certain state $\Phi = 0$. If substrate molecule binding takes place, the enzyme changes to state $\Phi = 1$ and consecutively to the states $\Phi = 2,3,\ldots$ etc., at regular time intervals Δt. In the particular state $\Phi = K_0$ at time $T_0 = \Delta t \cdot K_0$, the product molecule is now set free and the enzyme molecule undergoes further transitions $\Phi = K_0 + 1$, $K_0 + 2\ldots$ until at time $T = \Delta t \cdot K$ the last state $\Phi = K$ is reached. In the next step the enzyme molecule is once again set back to its initial state $\Phi = 0$. It is proposed that all reaction steps are irreversible.

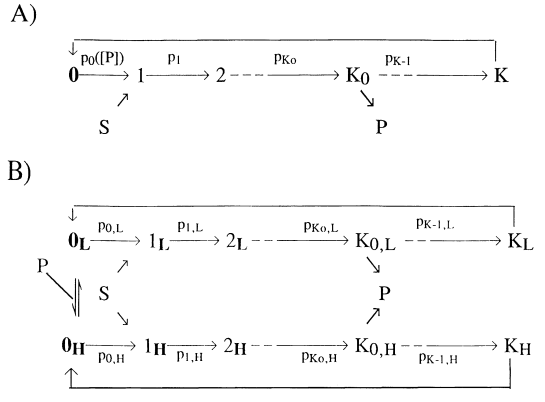

Fig. 1. Reaction scheme for (A) the model with variable binding probability or (B) model with distinct high and low affinity states. S is for substrate, P for product and the numbers symbolize the enzyme states $\Phi = 0,1,\ldots K_0,\ldots K$. The indices L and H are labels for low and high affinity states. The p_i are transition probabilities. For $p_i = k_{i,i+1}\Delta t = 1$ the transitions are deterministic, for $p_i = k_{i,i+1}\Delta t < 1$ are indeterministic.

In order to get a correlation between different reaction events an allosteric regulation mechanism is introduced: if bound by an enzyme, the reaction product is able to increase substrate affinity and, therefore, accelerates its own production. In the initial state $\Phi = 0$ the probability p_0 of capturing a substrate molecule depends on whether a product molecule is, or is not, bound to the regulatory site. If no product is bound, substrate binding probability is set to $p_0 = w_0$. Product binding at the allosteric site enhances affinity and the substrate binding probability is now set to $p_0 = w_1$. This probability w_1 is expressed as product of (i) the probability of collision between an enzyme molecule and a product molecule $\Delta t/t_{tr}$ during the time interval Δt, (ii) the low affinity substrate binding probability w_0 and (iii) an allosteric factor α, i.e. $w_1 = \alpha(\Delta t/t_{tr})w_0$. In fact, no explicit distinction is made between the high affinity and low affinity form of enzyme molecules; instead, a substrate binding probability $p_0 = 1 - (1 - w_0)(1 - w_1)^m$ is assumed for each enzyme molecule, with m as number of product molecules existing in the compartment.

In order to prevent all enzyme molecules from always being in the high affinity form, a first order decay with a rate γ is introduced, but, contrary to real decay processes, there is no distinction between free and bound product molecules. According to Mikhailov and Hess the mean lifetime of product molecules, $1/\gamma$, must be smaller than the recovery time $T - T_0$ of the enzyme [6]. In their opinion this choice is necessary to prevent immediate triggering of a new enzyme cycle by newly formed product molecules.

Hess and Mikhailov [4,6] report simulation runs of this process, where increasing the strength α of allosteric regulation forces the system from molecular chaos to periodic spiking in the number of product molecules.

A complication not taken into account by Hess and Mikhailov is the intrinsically stochastic nature of chemical reactions on the molecular level. As is emphasized in Refs. [4,6], the only indeterministic step is substrate binding. The subsequent transitions are assumed to be totally deterministic, so that the enzyme molecule works like a macroscopic clock or machine. Consequently, substrate turnover time is an exactly defined system parameter.

However, these reactions must show stochastic behavior since chemical reactions are of quantum mechanical nature. Even if they were of deterministic nature, coupling of molecules to the surrounding heat bath makes the behavior unpredictable. Hence, stochastic behavior, both with respect to binding of ligands and also to intramolecular reactions, has always been assumed in stochastic reaction kinetics since the beginnings (e.g. decay of Michaelis–Menten complexes in Ref. [8], a reference cited by Hess and Mikhailov).

Empirically, the indeterministic character of protein dynamics has been documented repeatedly in the vast number of single ion channel experiments (an introductory overview is given by Ref. [9]). [1] Another example which pertains directly to the present paper is given in Ref. [11]: Funatsu et al. measured turnover times of ATP in single myosin molecules by the imaging of single fluorescence labelled ATP molecules. The individual lifetimes of the ATP–myosin complexes showed considerable variation with an exponential distribution. The mean lifetime agreed well with the turnover time measured in solution. Hence, turnover time is a statistical concept.

2. Results

The original aim of the present analysis was to take into account the essentially stochastic nature of real enzymatic processes, expecting the persistence of (perhaps noisy) oscillations. Repetition of the Hess–Mikhailov simulations in the original manner [4] is very impressive if appropriate parameters are used, but a reproduction with stochastic models failed.

In stochastic reaction kinetics, first order reaction rates k_{ij} for transition from state i to state j are interpreted as decay probability per unit time (i.e. probability densities). The decay probability during a short time interval Δt is then given by $k_{ij}\Delta t$, provided $\Delta t \ll 1/k_{ij}$. If there is no branching in the reaction chain, survival probability in state i is $1 - k_{i,i+1}\Delta t$. Survival probability for a finite time interval $t = n\Delta t$ is then $(1 - k_{i,i+1}\Delta t)^n$, and the probability for a decay event between $(n-1)\Delta t$ and $n\Delta t$ is $k_{i,i+1}\Delta t(1 - k_{i,i+1}\Delta t)^{n-1}$. In order to get the usual continuous time variable one has to take the limit $\Delta t \to 0$ and $n \to \infty$, but in such a way that $t = n\Delta t =$ constant.

On paper this process is straightforward, but in computer simulations this operation is not possible, and instead one has to choose Δt as small as possible. But with small enough values for Δt, memory limitations come into play. For these reasons, smaller time steps Δt imply that simulated time intervals are of shorter duration and a compromise most be found.

Deterministic processes can be implemented if $k_{i,i+1}$ is chosen in such a way that $k_{i,i+1} \cdot \Delta t = 1$, implying a 100% transition probability during Δt. In this case, a small complication cames from the fact that if Δt is decreased, the number of deterministic states Φ must be increased.

The following parameter set from Ref. [4], leading to coherent spiking in the deterministic case was chosen as reference for the investigations discussed below: low affinity substrate binding rate 0.01 ms^{-1} enzyme $molecule^{-1}$, allosteric factor $\alpha = 70$ (corresponding to a high affinity binding rate of 0.07 ms^{-1} enzyme $molecule^{-1}$), and transition rates $k_{i,i+1} = 1$ ms^{-1} enzyme $molecule^{-1}$ for each transition $\Phi = i$ to $\Phi = i + 1$ $(i > 0)$ with a simulation time step $\Delta t = 1$ ms. For the given Δt this corresponds to deterministic transitions after each 1 ms step, i.e. a transition probability of 100%, as mentioned above. There are 21 states Φ with $0 \le i \le 20$ and release phase $K_0 = 10$. The product decay rate is set to $\gamma = 0.3$ ms^{-1}, corresponding to a mean lifetime of 3.3 ms. with these given parameters, spikes are clearly seen at intervals of about 10 ms (Fig. 2A). A mean turnover time of $t_{\text{turnover}} = 21.68 \pm 1.05$ ms is obtained for the simulation given in Fig. 2. In order

[1] See especially Ref. [10].

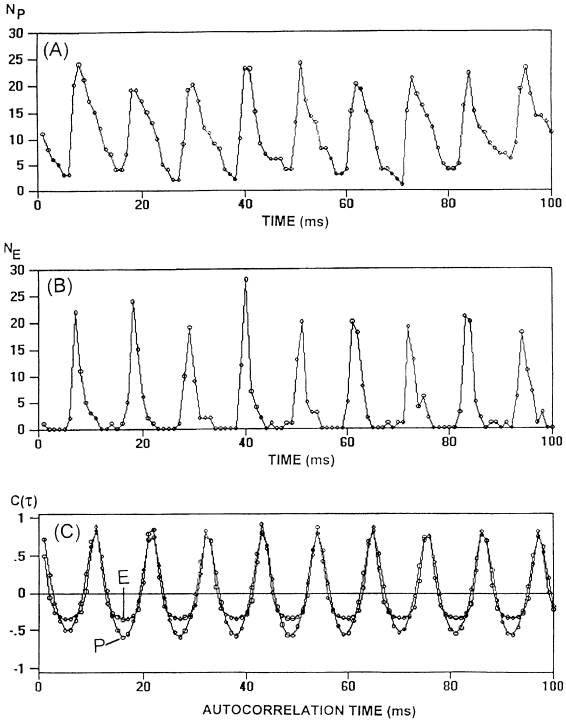

Fig. 2. Deterministic Hess–Mikhailov process with $\Delta t = 1$ ms. Parameters as given in the text. (A) Number N_P of product molecules vs. time and (B) number of enzyme molecules N_E in product releasing state $K_0 = 10$ vs. time. (C) Autocorrelation function $C(\tau) = <(N(t) - \langle N \rangle)(N(t+\tau) - \langle N \rangle)/\langle N \rangle^2$ of number of product (P) and enzyme (E) molecules in product release state $K_0 = 10$ (E). All 100 ms traces are parts of 500 ms simulations recorded during the stationary reaction phase.

to exclude initial transient effects, several consecutive simulation runs, each lasting 500 ms, are performed before analyzing the last one. The figures show a 100 ms part of such 500 ms traces. Number of product (Fig. 2A) and enzyme molecules in state K_0 (Fig. 2B) show periodic temporal variation with a periodicity half the length of the turnover time. This periodicity is also seen in the autocorrelation function of the number of product molecules or the number of enzyme molecules in state $\Phi = K_0 = 10$ (Fig. 2C). A histogram of the distribution of phases Φ for an arbitrarily chosen time also showed clustering around two maxima 10 ms apart, as seen in the original paper [4] (data not shown). Interestingly, different sets of simulation runs show differences in spike shape, which persist during the whole experiment. It seems that the system might have some form of memory.

In order to introduce stochastic transitions (Fig. 1A, with $p_i < 1$), a transition probability density (or first order reaction rate) of $k_{i,i+1} = 1\ \mathrm{ms}^{-1}$ for all steps $i > 0$ was chosen (as in the case above), but with time steps Δt usually set to 0.1 ms. The transition probabilities are then $k_{i,i+1}\Delta t = 0.1$ during the time interval Δt. The probability of finding a molecule in its initial state after 1 ms is therefore $(1 - k_{i,i+1}\Delta t)^{10} = (1 - 0.1)^{10} = 0.348\ldots$. In comparison, a continuous physical process shows a survival probability of $\exp(-k_{i,i+1}(10\Delta t)) = \exp(-1) = 0.367\ldots$. Hence the former mode gives an error of about 5%. Smaller time steps would show essentially the same behavior and the approximation to the physical process would be better, but as mentioned above test runs with smaller time steps have the disadvantage of computer memory limitations, allowing simulations only over shorter time scales.

Under the conditions given above, the mean turnover time and standard deviation increase to $t_{\mathrm{turnover}} = 26.57 \pm 8.37$ ms. Time course of the total number of substrate molecules (Fig. 3A) or enzyme

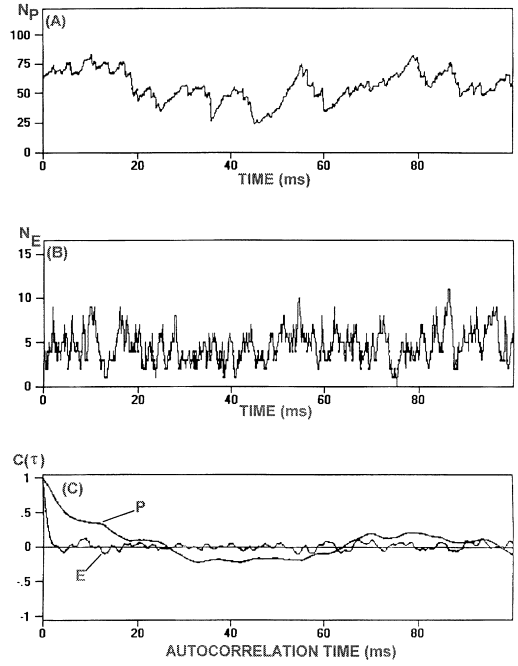

Fig. 3. The same as in Fig. 2, except the time step $\Delta t = 0.1$ ms, and therefore indeterministic behavior.

molecules in the 'product-firing' state K_0 (Fig. 3B) now do not show any temporal regularity. Autocorrelation (Fig. 3C) and Fourier analysis (data not shown) likewise show no periodicity. In the phase histogram periodicity is also lost (data not shown).

In the original Hess–Mikhailov model no distinction was made between the high and low affinity form of the enzyme. Instead each enzyme molecule was able to bind a substrate molecule with the same probability p_0. To see whether distinguishing the two forms, and taking their interplay into consideration might restore oscillating activities, the model was extended in the present study (Fig. 1B).

A second order rate was introduced for regulatory product binding to the low affinity form to transform the low to the high affinity species. Product binding enables transformation to the high affinity species. In Refs. [5,6] binding probability depends on the number of currently existing product molecules. Applying this assumption to the two affinities model means very fast, i.e. diffusion controlled binding. Therefore a product binding rate of $k_{\text{binding}} = 0.1\ \text{ms}^{-1}$ enzyme molecule^{-1} product molecule^{-1} (the inverse of traffic time t_{tr}) was chosen. Since in the original model product molecules were eliminated irrespective of binding, dissociation from the regulatory site must be faster than elimination. In the simulations done with the two affinities model only free molecules are eliminated at rate γ. Product dissociation from the allosteric binding site was varied from $k_{\text{diss}} = 0.01$ to $1.0\ \text{ms}^{-1}$ enzyme molecule^{-1}, because no hint was available for a reasonable value.

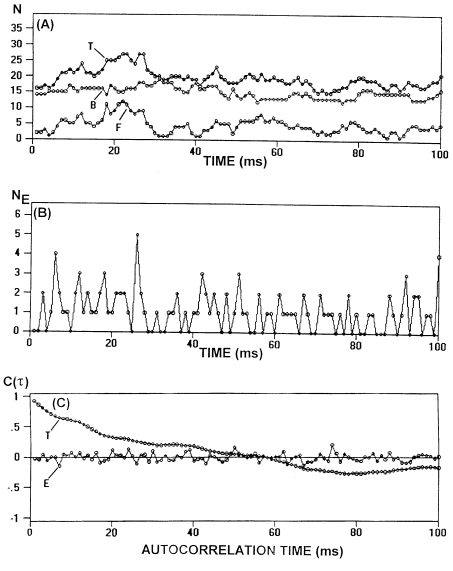

Fig. 4. Deterministic two affinities model, parameters are chosen as close as possible to the original model (see text). (A) Time course of total (T), bound (B) and free (F) product molecule number N. (B) Number N_{E} of enzyme molecules in state $K_0 = 10$. (C) Autocorrelation of enzyme (E) and total product (T) molecule number.

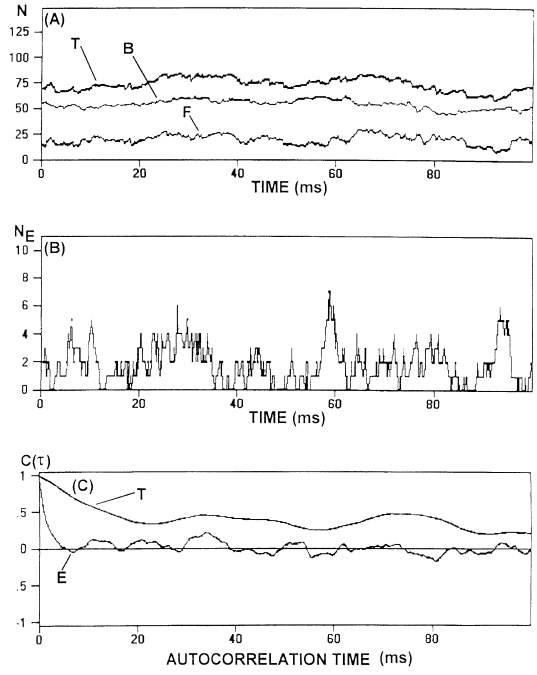

Fig. 5. Two affinities model with indeterministic transition (Δt = 0.1 ms); otherwise conditions as in Fig. 4.

An extensive search for periodic behavior in the deterministic two-affinities model failed. Even on attempting to choose conditions matching those of the original model as closely as possible, i.e. employing the same transition rates, diffusion controlled binding and varying release rates for bound effector product molecules, no periodicity is seen (Fig. 4). Moreover, it was not possible to get a short turnover time; for example, the turnover time was $t_{\text{turnover}} = 83.98 \pm 87.97$ ms for $k_{\text{diss}} = 0.1\ \text{ms}^{-1}$.

Accordingly, it is not surprising that for the indeterministic case (with $\Delta t = 0.1$ ms) no periodic behavior is seen (Fig. 5).

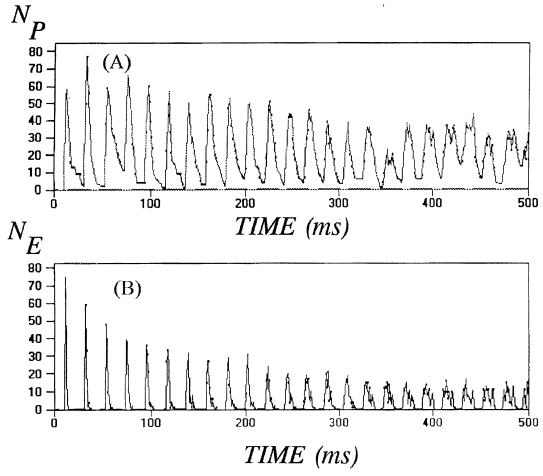

Fig. 6. Deterministic transition ($\Delta t = 1$ ms); reaction chain with only one affinity state. Binding rate $k_0 = 0.8$ ms^{-1}. All other steps are deterministic ($k_{i,i+1} = 1$), product release in state $K_0 = 10$ as before. System shows oscillations caused by the initial binding conditions. (A) Time dependence of product number. (B) Number of enzyme molecules in releasing state.

Under all investigated conditions mean turnover time and standard deviation were of the same order of magnitude, allowing no synchronization of the enzyme molecules.

These results suggests that oscillations have something to do with the 'stiff' behavior of the Hess–Mikhailov model. Hence, other deterministic models should also show similar types of oscillations, as illustrated by two 'pathological' variations of the deterministic model.

The first model is given by the following conditions (Fig. 6): (1) A reaction cycle with 21 states and release state $K_0 = 10$, as above, (2) only one indeterministic substrate binding state without allosteric regulation, (3) all enzyme molecules at time $t = 0$ are in the same state $\Phi = 0$, (4) transitions are deterministic and $\Delta t = 1$ ms, (5) $\gamma = 0.3$ ms^{-1}, as above. Substrate is bound with high affinity, but without allosteric regulation ($p_0 = w_0 = 0.8$ ms^{-1}, $\alpha = 0$ and $w_1 = 0$). The initial fast substrate binding burst synchronizes enough enzyme molecules to get oscillations of about 21 ms duration. Since the first reaction step is indeterministic, molecules should slowly run out of phase and the originally high spikes decrease with time, but they are clearly seen for a long time. Decreasing w_0 to 0.6 ms^{-1} leads to initial oscillations which vanish within 1000 ms (data not shown); if w_0 is further decreased to 0.4 ms^{-1} no oscillations are seen.

With this phenomenon in mind, a variation of the original Hess Mikhailov model as second model is interesting. Setting all parameters as in the reference model, except $\gamma = 0$ (i.e. no product elimination), shows the following effect: the number of product molecules rises in a stepwise fashion with a step length of about 21 ms (Fig. 7A). The majority of enzyme molecules is synchronized and shows spikes for the phase K_0 at the beginning of new substrate steps (Fig. 7B).

This can be explained in the following way: at time zero, some enzyme molecules are recruited into phase $\Phi = 1$ with probability w_0. This means that after the first 10 ms the number of product molecules (on average) increases linearly for the next 10 ms. After this time, non-recruited enzyme molecules are able to bind product molecules and since the allosteric mechanism increases substrate binding affinity in a non-linear way, enzyme recruitment increases faster than product making. A short time later, all enzyme molecules are in an activated state $\Phi > 0$ and new product molecules cannot increase enzyme activity further. Hence the phase histogram has a triangular appearance, showing that most enzyme molecules cluster around certain states. Since the number of product molecules after a short time is

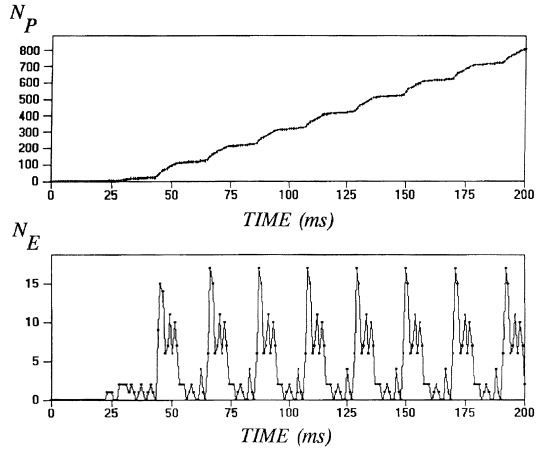

Fig. 7. Deterministic transitions like in Fig. 2, but without product decay ($\gamma = 0$). (A) Increase in product number. (B) Time course of enzyme molecules with $K_0 = 10$.

as big as, or greater than, the number of enzyme molecules ($m = N_C = 100$; to be consistent, one has to set $m = N_C$ in the formula for p_0 when the number of product molecules exceeds the number of enzyme molecules), binding probability for substrate molecules is nearly 100% ($p_0 \approx 1$). Thus, even the substrate binding step becomes deterministic, and the system 'remembers' its initial transient behavior and repeats a recruitment scheme very similar to the first one (Fig. 7).

From this standpoint the function of product elimination in the reference model is only to differentiate (in the mathematical sense of calculus) the output signal of the enzyme system. It is not necessary that product molecules have a mean life time $1/\gamma < T - T_0$, as demanded by Mikhailov and Hess [6]. It is even possible to get oscillations in the reference model if the only modification is that $K_0 = K$, i.e. if the product is set free in the last enzyme state. It might appear from the above arguments that product elimination is unnecessary, but this is not true. If enzyme molecules in the beginning are in different states Φ, product elimination at an appropriate rate γ synchronizes the system.

3. Discussion

A closer look at the Hess Mikhailov model shows that there are three main factors causing oscillatory behavior. Firstly, the only indeterministic step in the model is substrate binding. All other transitions from states $\Phi = i$ to $\Phi = i + 1$ are fully deterministic (i.e. the transition probability is 100%), but in a true enzyme molecule they are probabilistic. The 'stiff' behavior prevents desynchronization of different enzyme molecules. Secondly, the allosteric process was simplified: the total probability for substrate binding is set to $p_0 = 1 - (1 - w_0)(1 - w_1)^m$. This formula suggests that each new product molecule is instantaneously bound to the regulatory site of an enzyme molecule. In the more realistic case of distinct low and high affinity forms, the regulatory molecules must be bound during a finite time interval. Here an upper limit for binding rates is given if diffusion controlled reactions are assumed. This binding process is of a magnitude comparable to the transitions between different enzyme states. Thirdly, product molecules decay at first order rate γ, without distinction as to whether they are free or bound. This suggests fast dissociation, since usually only free molecules are accessible to degrading enzymes. The overall effect of product decay is one of resynchronization, since product molecules are removed between two collective release events. Moreover, decay shapes the product spikes (increasing γ makes thinner spikes).

In stochastic reaction kinetics, turnover time t_{turnover} (even maximal turnover time for saturating substrate concentrations) is a statistical measure. It is the mean duration of a reaction cycle. Therefore there is no guarantee that a set of synchronized enzyme molecules will stay synchronized. Preservation of synchronization for longer times demands very small dispersion as compared to turnover time.

Mean turnover time for an irreversible cycle with $n + 1$ states $0 \le i \le n$ is given by $t_{\text{turnover}} = 1/k_0 + 1/k_1 + \ldots + 1/k_n$. It is difficult to produce the accompanying formula for dispersion σ and it is even more difficult to write down t_{turnover} and σ for a reversible process. However for irreversible processes it is possible to discuss two enlightening special cases after consideration of a preliminary example.

Consider the decay of a molecule A into a molecule B in an irreversible first order reaction: $A \rightarrow B$. This process is characterized by a rate constant k with dimension time^{-1}. The number of molecules $N_A(t)$ existing at time t is determined by $N_A(t) = N_A(0)\exp(-kt)$. In stochastic reaction kinetics k is interpreted as the decay probability per unit time. Decay probability during a short time interval Δt is therefore $p_{\text{dec}} = k\Delta t$. The probability of A not decaying is $p_{\text{not}} = 1 - k\Delta t$. After the longer time interval $t = n\Delta t$ the following probabilities apply:

$$\text{probability}\{\text{A exists at time } n\Delta t\} = (1 - k\Delta t)^n$$

$$\text{probability}\{\text{A decays between } (n-1)\Delta t \text{ and } n\Delta t\} = k\Delta t(1 - k\Delta t)^{n-1}$$

In the case of limit $\Delta t \rightarrow 0$ (with $t = n\Delta t =$ constant), from the formula for probability{A exists at time $n\Delta t$} it follows:

$$\text{probability}\{\text{A exists at time } t\} = \exp(-kt).$$

From the above formulae it follows for mean $\langle t \rangle$

and square mean $\langle t^2 \rangle$ life time

$$\langle t \rangle = \sum_{n=0}^{\infty} (n\Delta t) k\Delta t (1 + k\Delta t)^{n-1}$$

$$\langle t^2 \rangle = \sum_{n=0}^{\infty} (n\Delta t)^2 k\Delta t (1 - k\Delta t)^{n-1}$$

After some simple algebraic manipulation one gets

$$\langle t \rangle = \frac{1}{k},$$

$$\langle t^2 \rangle = 2\langle t \rangle^2 - \langle t \rangle \Delta t = \frac{2}{k^2} - \frac{\Delta t}{k}$$

and for dispersion

$$\sigma^2 = < (t - \langle t \rangle)^2 > = \langle t^2 \rangle - \langle t \rangle^2 = \frac{1}{k^2} - \frac{\Delta t}{k}.$$

For $\Delta t \to 0$ one gets the usual formulae for mono-exponential decay with $\langle t \rangle = 1/k$, $\langle t^2 \rangle = 2/k^2$ and $\sigma^2 = 1/k^2$, i.e. it is always $\sigma^2 = O(\langle t \rangle^2)$. These relations also show the influence of finite Δt and digitalization in computer simulations. Interestingly, dispersion is smaller in the digitalized version of the problem, since processes with a time scale smaller than Δt are suppressed. A very important fact is that there is no freedom in choosing dispersion or the stochastic noise, since both are fully determined by k.

Now it is possible to discuss the two special cases mentioned above: in the first case, only one rate limiting step k_{limit} exists (e.g. if low substrate binding rate and fast transitions between consecutive intermediate states are given). In this situation $t_{\text{turnover}} \approx \langle t \rangle \approx 1/k_{\text{limit}}$ and σ depend primarily on k_{limit} similar to the situation in first order decay $A \to B$ discussed above. Hence $\sigma^2 = O(t^2_{\text{turnover}}) = O(1/k^2_{\text{limit}})$ as above, and an originally synchronized set will quickly desynchronize.

In the second special case there are n rate limiting steps of about equal rate (e.g. if very fast binding is followed by n rate limiting transition steps of equal magnitude). The probability density for the transition between step i and step $i+1$ is always given by $P_{i \to i+1}(t) = k \cdot \exp(-kt)$. Probability density $P_{i \to i+2}(t)$ for the transition from i to $i+2$ via $i+1$ is given as the convolution integral:

$$P_{i \to i+2}(t) = \int_0^t \mathrm{d}t' P_{i \to i+1}(t') P_{i+1 \to i+2}(t - t').$$

Repeating this process, one gets similar formulae for longer reaction chains. Inserting the expressions for $P_{i \to i+1}$, the probability density function for running through the full cycle $0 \to 1 \to 2 \to \ldots \to n \to 0$ exactly once during time interval t is

$$P_{\text{cycl}}(t) = \frac{k^{n+1} t^n}{n!} e^{-kt}.$$

The qth statistical moment of time (e.g. $q = 1$ mean time $\langle t \rangle$, $q = 2$ square mean time $\langle t^2 \rangle$) is given by

$$\langle t^q \rangle = \int_0^{\infty} \mathrm{d}t P_{\text{cycl}}(t) t^q = \frac{(n+q+1)!}{n! k^q}.$$

In particular, one gets $\langle t \rangle = (n+1)/k \approx t_{\text{turnover}}$ and $\langle t^2 \rangle = (n+1)(n+2)/k^2$. For dispersion $\sigma^2 = (n+1)/k^2$ follows.

Thus, with increasing n, the ratio $\sigma : t_{\text{turnover}}$ decreases with $1/\sqrt{(n+1)}$. For $n \approx 20$, one gets the not so unreasonable value $\sigma \approx t_{\text{turnover}}/4$ since for the indeterministic version of the Hess Mikhailov model we got $\sigma \approx t_{\text{turnover}}/3.17$, i.e. it is also $\sigma^2 = O(t^2_{\text{turnover}})$ as before. Only for very large values of n the dispersion σ is much smaller than t_{turnover}. For more complicated cases the situation must be similar, but with factors leading to further increase in σ.

4. Conclusion

In summary, Hess and Mikhailov's model works only due to its deterministic and non-linear character. The strong spatial correlation is a necessary, but per se not a sufficient condition to obtain synchronization of different reaction events. This is also true for the non-linear oscillator models cited in Ref. [4] as analogues. Contrary to these suppositions the quantum mechanical nature of chemical reactions and the coupling of enzyme molecules to the surrounding heat bath demand indeterministic behavior, and indeterministic behavior is, indeed, observed in experiments with single protein molecules. When for these reasons their model was extended by introducing stochastic transitions between different enzyme states, the coherent behavior of the enzyme collective was destroyed. As shown by discussion of turnover times as a function of their respective reac-

tion rates, dispersion of individual cycling times is of the same order of magnitude as the turnover time. This prevents the possibility of resynchronization, which is a prerequisite for coherent behavior.

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