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Biophysical Chemistry 71 (1998) 83–85

Biophysical Chemistry

Fluctuations of molecular turnover times in enzymatic reactions: reply to W. Wyskovsky

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Keywords: Biochemical reactions; Molecular turnover times; Molecular networks

1. Introduction

Recently, we have published a series of articles [1–5] devoted to theoretical investigations of biochemical reactions in small spatial volumes. Our estimates show that in the volumes of micrometer and submicrometer sizes, all characteristic kinetic times, associated with diffusional transport of molecules through the volume to their respective targets, may become much shorter than characteristic dynamic molecular times, related to conformational transformations in the involved macromolecules. This implies that the reaction can no longer be modelled as a sequence of instantaneous reaction events with vanishing durations. Instead, each molecular reaction event should then be temporally resolved as a continuous dynamical process, and thus physics of intramolecular relaxation and comformation transitions should become an unavoidable element of the theoretical description. We have suggested to classify biochemical systems, where these conditions are satisfied, as *molecular networks* [1,5].

2. Discussion

Direct fast coupling of molecular dynamics can lead to emergence of strong correlations between dynamical states of macromolecules in a small volume. To illustrate this, a simple model of an enzymatic reaction with allosteric product activation has been considered [4,5]. In this model, the dynamics of continuous conformational changes during a single turnover cycle of an enzyme molecule has been treated as deterministic motion along a cyclic phase coordinate. Our numerical simulations [4,5] and analytical studies of the mean-field limit of this model [6] have revealed that, when the intensity of allosteric regulation exceeds a certain threshold, synchronization of single molecular cycles of different enzymes is found.

In his Comment [7], W. Wyskovsky argues that synchronization will always be absent when fluctuations inside molecular cycles are taken into account. We cannot accept this conclusion, as explained below.

Indeed, already in the original version of the model [4,5], some fluctuations in the effective duration of single molecular cycles were present since binding of a substrate molecule and, hence, initiation of a cycle represented stochastic events with a cer-

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PII S0301-4622(98)00092-1

tain temporal dispersion $\Delta t_{\text{binding}}$. If dispersion Δt_{turn} of turnover times, caused by fluctuations inside the cycle, is less than $\Delta t_{\text{binding}}$, its effects are negligible.

We have also performed detailed statistical investigations of an extended model, taking additionally into account fluctuations inside the cycle. Our data, collected over a large number of simulations with varying reaction parameters, shows that synchronization persists in this system even at larger dispersions of turnover times, i.e., when Δt_{turn} is up to 20% of t_{turn}, depending on the parameter choice [8]. Moreover, the synchronization behaviour can well be found when different assumptions about the details of allosteric regulation are taken.

The conclusion of W. Wyskovsky is actually based, as seen from the Comment [7], on only two sets of simulations with $t_{\text{turn}} = 26.57 \pm 8.37$ ms and (for a modified model) with $t_{\text{turn}} = 83.98 \pm 87.98$ ms. Evidently, these two specific simulations with strong statistical dispersion of turnover times do not and cannot imply that the synchronization is *always* destroyed by turnover cycle fluctuations.

To support his conclusion, W. Wyskovsky further claims that, on general theoretical grounds, the dispersion of individual cycle times *must* be of the same order of magnitude as the turnover time. This claim, however, contradicts even his own analysis in the discussion section of the Comment. Indeed, by picturing the cycle as a sequence of n irreversible probabilistic transitions, each at an equal rate k, he finds that the relative dispersion behaves as

$$\frac{\Delta t_{\text{turn}}}{t_{\text{turn}}} = \frac{1}{\sqrt{n+1}}. \qquad (1)$$

Hence, it should become very small when the number n of intermediate states inside the cycle is large. Paradoxically, this calculation actually shows that our original deterministic model, where fluctuations inside the cycle are neglected, is exactly recovered when the limit $n \to \infty$ is taken. Thus, unless W. Wyskovsky knows some universal upper limit on the number n of intermediate states inside the cycle, his own analysis rather supports the opposite statement, i.e., that the fluctuations *may* be relatively weak under certain conditions.

Though logically inconsistent, this last argument shows, additionally, that W. Wyskovsky does not adequately understand our model. His picture of the molecular enzyme dynamics is that it rather represents a sequence of sudden jumps between a few discrete physical states, such that the overall speed of motion along the cycle is determined by waiting times for the transitions between these discrete states, whereas the duration of every single jump is so short that it can be neglected. This picture has indeed been employed in the old models of enzyme kinetics proposed by Bartolomey [9].

Subsequent theoretical and experimental research on physical properties of proteins, representing large heteropolymer molecules, have, however, brought essential modifications to the understanding of molecular enzyme dynamics. The protein molecules are now known to have very complex internal dynamics, such that their equilibrium and kinetic properties can be similar to those of spin glasses [10,11]. Usually, they would have a great number of quasi-equilibrium states, corresponding to local minima of free energy and separated by energy barriers of varying heights. The kinetics of configuration changes and of conformational relaxation in such macromolecules is best approximately described as an effective diffusive biased motion along a continuous collective conformational coordinate [12,13]. The characteristic time of conformational changes in protein molecules is typically in the range of 0.01 s (although folding of proteins can be even a much more longer process).

A turnover cycle of an enzyme molecule represents a combination of conformational changes, bringing substrate molecules to the optimal positions inside a substrate–enzyme complex, and of an electronic transition leading to formation of new chemical bonds and emergence of a new product molecule. Therefore, the turnover time is generally determined by the duration of needed conformational changes plus the waiting time for an electronic transition when an optimal configuration is reached. Although the turnover times may greatly vary, for many enzymes they are given by several hundredths of a second. It means that conformational changes would essentially contribute in this case to the total turnover time of the catalytic molecular cycle.

If the catalytic activity of an enzyme is high, the waiting time for a final electronic transition is relatively small. Neglecting it, the molecular cycle dy-

namics is represented as an operation of a microscopic molecular machine, based on the conformational changes inside protein macromolecules and driven by the energy brought with the substrate [14–16]. It is this concept that has been implemented in our simple model [4,5]. Respectively, our phase is in reality a continuous variable and only after a mathematical discretization, we arrive at a formulation where discrete phase states appear. This means that, for us, the total number n of phase states is only an artificial discretization parameter that ideally should be taken very large. The value of $n = 20$ was chosen in Refs. [4,5] only for convenience; similar results are obtained in our model when much larger values up to a thousand are taken [8,17]. We note that a more adequate modelling of the enzymic cycle is obtained assuming that the phase is a continuous variable, whose dynamics is governed by a stochastic Langevin equation that includes both a diffusive drift in a jagged biased potential and a noise term. Such modifications are formulated and studied in our forthcoming publication [8].

The question of turnover time fluctuations in enzyme molecules is a complex problem that can only be theoretically investigated by considering real physical processes in particular protein molecules. It is also an interesting experimental problem, especially taking into account recent significant progress in single-molecule studies [18]. The intensity of fluctuations may depend on the experimental parameters and the choice of an enzyme. Recent experimental investigations [19] of the cytochrome P-450-dependent mono-oxygenase system have revealed that in this case the cycle contained approximately 40–60 individual steps and it could well be approximated by a continuous model of the diffusive biased motion. The experiments [19] involved synchronization of enzymic cycles by external applied signal. The measured memory time of the system corresponded to the statistical dispersion of cycle times of about 20%.

3. Conclusion

Our own theoretical investigations have so far been focused on the conceptual aspect, intending to attract attention to possible new kinetic properties of biochemical reactions in small spatial volumes. We have done this by providing simple estimates of characteristic kinetic properties of such systems and by proposing simple skeleton models. Clearly, there is still a large distance between such models and the actual enzyme reactions. It has also never been said by us that the predicted synchronization behaviour should occur in *any* enzyme population under *any* conditions. Only further theoretical and experimental studies, including formulation and analysis of more detailed mathematical models, can answer the question to what extent synchronization of individual molecular cycles is realized in nature.

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