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ASPECTS OF FRACTAL KINETICS OF ENZYMATIC REACTIONS BY MONTE CARLO SIMULATIONS

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SUMMARY

Using Monte Carlo simulations in 3D media we investigate the effect of macromolecular crowding on biochemical reactions following a Michaelis-Menten kinetics. In the system substrate and product particles cannot overlap and the effect of crowders mobility and concentration are examined. The simulation data are analyzed in terms of parameters describing the time dependence of the rate coefficient. Our results indicate a fractal like kinetics with different degrees of fractality depending on crowders features. Even though small, when crowders mobility rises kinetics fractality decreases due to enhancement of diffusional movements of the reactants. Instead, increasing the crowders density in the system kinetics fractality presents a smooth growth as less free volume is available for reactants.

Keywords: fractal kinetics; enzymatic reactions; Monte Carlo simulation; crowding.

INTRODUCTION

There is an increasing interest in the literature of the last years for simulations of enzymatic reactions taking place in crowded environments [1-3]. These reactions have an overwhelming importance for living organisms and belong to diffusion mediated processes.

Molecular crowding is relevant to cells because they contain high concentrations of biological macromolecules. The great diversity of these molecules complemented by their diffusion coefficients correlated with molecular weight gives the "picture" of a net filled with diffusive particles of various dimensions and mobility. In this context, each particle is perceived as an obstacle by any other particle of the system hindering molecular motion Diffusion is thus affected and biochemical processes are significantly altered [4-6].

Experimental studies have shown that kinetics of processes developing in heterogeneous and crowded media cannot be explained anymore by mass action law due to anomalous, time dependent behavior of the rate constant. For obstructed systems, the linear decrease of rate constant with time in double logarithmical coordinates has been revealed. It has become improper to further use the term of rate constant, thus being replaced with that of rate coefficient [7].

Kopelman (1988) proposes a new description of reaction kinetics in crowded media, so-called fractal kinetics. This implies that reaction occurs similarly on different time scales. Accordingly, time dependence of the rate coefficient is described by the following equation:

$$k(t) = k_0 \cdot t^{-h} \qquad (1)$$

where k_0 is the rate constant in classical kinetics, $t > 0$ time and $0 \le h \le 1$ a constant named fractal parameter, a measure of system dimensionality (system molecular crowding).

This equation has been proposed to describe kinetics of all diffusion controlled reactions (reaction order ≥ 2). For unobstructed systems, the fractal parameter is null and kinetics is classical. However, the rate equation formalism does not work for $t \to 0$ and $h > 0$ case in which $k(t) \to \infty$. This model should be valid for any temporal scale, then for $t = 0$, too.

A solution has been proposed by Schnell and Turner (2004) considering a fractal kinetics of which rate coefficient follows a Zipf-Mandelbrot distribution:

$$k(t) = \frac{k_0}{(\tau + t)^h} \quad 0 \le h \le 1, \qquad (2)$$

Parameters k_0 and h have the same significance in this case as in Kopelman equation (1) and τ is a positive constant that represents the time after which reaction is influenced by molecular crowding [2].

These theories should be validated by experimental approaches. However in *vivo* studies of biochemical processes are difficult to perform. Computational simulations have been proved to be essential in overpassing these limitations. Using Monte Carlo algorithms, complex systems have been designed and different conditions imposed to better model real

situations encountered in living organisms. New evidences have been brought that in crowded environments diffusion is anomalous for short periods of time and rate coefficient of enzymatic reactions is time dependent [1-3, 8-12]. Diffusion anomaly and kinetics fractality are stronger as the medium is more crowded, molecules are immobile and their dimensions smaller [13].

Berry (2002) studied the dynamics of Michaelis-Menten reaction using a Monte Carlo algorithm for a two dimensional lattice with cyclic boundary conditions. Immobile obstacles have been randomly distributed with a density varying from zero to the percolation threshold (for a 2D lattice percolation threshold is reached for an obstacle concentration of $[O]_P$=0.4073). His studies have shown a fractal kinetics as a result of both reduced dimensionality of the system and excluded volume induced by molecular crowding. Fractal features of enzymatic kinetics have been quantitatively assessed by fractal parameter *h* from Kopelman's equation. The parameter increases with the rise of obstacle and substrate concentrations, their individual effects being mostly cumulative.

The algorithm proposed by Berry had been later used in similar conditions by Schnell and Turner (2004). Besides confirming the time dependence of k_1, they had revealed a better description of temporal behavior of rate coefficient using a Zipf-Mandelbrot distribution (Eq. 2). The errors of nonlinear fitting of simulation data were significantly smaller than those obtained using Kopelman's equation (1). On the other hand, they had extended Berry's algorithm for MM processes in 3D lattices without obstacles identifying a classical kinetics, in very good accordance with theoretical predictions.

In other studies, the same working group has revealed that intracellular reaction dynamics can be described both by fractal kinetics and mass action law [10, 11, 14]. The key factor of this distinct behavior is reaction probability. Moreover, the geometry of intracellular space does not alter the law describing reaction kinetics. As a result, laws describing chemical reactions in intracellular media are different than those occurring in heterogeneous inorganic ones.

Other groups [3, 4, 12] have analyzed some computational aspects of Berry's algorithm implementation in 2D and 3D crowded media, with obstacles of various dimensions and mobility. Particularly, specific methods of obstacles and reactants distribution in lattice have been considered and also the effects produced by all eight neighbors of each particle instead of four previously used.

For the moment, there are no experimental evidences certifying temporal dependence of rate coefficients of diffusion mediated processes. The only experimental proofs refer to smaller diffusion coefficients of reactants in crowded media than in homogeneous ones. Also, it is well known the rate coefficients dependence on diffusion coefficients [15].

The present study comprises Monte Carlo simulations of enzymatic processes in crowded media following a Michaelis-Menten kinetics, performed in order to explore and bring more insights of the fractal behavior of the rate coefficient.

The paper aims at analyzing temporal dependence of k_1 by simulations of the following cases:

A) the validation of simulation for a 3D lattice (100x100x100) filled only with

reactants with different initial concentrations ([S]=0.1, [E]=0.01; [S]=0.2, [E]=0.01; [S]=0.2, [E]=0.02);

B) the comparison of enzymatic kinetics taking place in 2D and 3D crowded media;

C) the effect of obstacle mobility in lattices with identical obstacle density ([O]=0.37), enzyme and substrate concentration ([E]=0.01, [S]=0.2).

MATERIALS AND METHODS

For this study we have programed in Fortran 77 an algorithm modifying the one of Berry [1] by considering 3D cubic lattices and obstacles with different degrees of mobility.

The simulation system contains five types of particles: enzyme (E), substrate (S), complex enzyme-substrate (C), product (P) and obstacles (O). The obstacles are nonreactive species that moves or not into the lattice and they are used to generate molecular crowding. All the other particles are mobile and their mobility is modeled by random diffusional movements. The model of rigid-sphere repulsions is considered and thus two particles cannot occupy simultaneously the same position in the lattice. Rate coefficients are represented by reactions probabilities with similar values used in the literature: f=1 for k_1, r=0.02 for k_{-1} and g=0.04 for k_2, respectively [1,2].

Then, using a Monte Carlo procedure to generate random sampling, one mobile molecule is chosen to move or react, depending on its type, and also its new position. In a 3D lattice the 6 neighboring positions are considered. The simulation algorithm we followed is described in detail in another article [15].

In a real system all particles are moving at the same time. Therefore, the simulation temporal step t represents the repetition of the Monte Carlo sequence by $N_{tot}(t)$ times ($N_{tot}(t)$ is the total number of mobile particles in the system at time t). This, at least statistically, assures the movement of each particle in a temporal step. Each simulation has between 800 and 1000 steps and the values calculated for the reactants concentration and rate coefficients are mediated over 200 and up to 500 independent runs. The reactants concentrations are given by their corresponding densities, calculated as the ratio between the number of particles and total number of the lattice positions. The obstacle concentration is under percolation level for all simulations performed.

The effects of immobile (M=0) and mobile (M=1) obstacles are considered. Their mobility (M) is given by their probability of movement, for which 0 and 1 values have been attributed. Every time an obstacle is randomly chosen to move, a number between 0 and 1 is generated and compared with the movement probability. If this number is smaller than the probability then particle moves to the new position.

The simulation data consist of concentration of all species at each time step: [S](t), [E](t), [C](t) and [P](t). The number of collisions enzyme-substrate conducting to reaction is also recorded [1]:

$$\gamma(t) = \int_0^t k_1(t)[E](t)[S](t)dt \qquad (3)$$

allowing to determine the rate coefficient k_1

$$k_1(t) = \frac{1}{[E](t)[S](t)} \frac{d\gamma(t)}{dt} \qquad (4)$$

The k_1 expression is then used in the kinetic equations describing the Michaelis-Menten mechanism and the expressions of the other two rate constants are obtained:

$$k_{-1} = \frac{1}{[C](t)} \left(\frac{d\gamma(t)}{dt} + \frac{d[S](t)}{dt} \right) \qquad (5)$$

$$k_2 = \frac{1}{[C](t)} \frac{d[P](t)}{dt} \qquad (6)$$

All temporal variations of reactants concentration and enzyme-substrate collisions appropriate for reaction are calculated using the first order approximation:

$$\frac{dx}{dt} = \frac{x(t) - x(t - \Delta t)}{\Delta t} \qquad (7)$$

In order to analyze the kinetics fractality the coefficients of equation (1) are calculated. The Levenberg-Marquardt procedure is used to linearly fit the simulation data with equation (1) under the Origin 7.5 package (OriginPro, OriginLab Corp.).

RESULTS AND DISCUSSIONS

A) The validation of simulation algorithm

In a three dimensional medium diffusion might be a process of perfect mixing and, in the absence of obstacles, the reaction kinetics must be classical. Therefore, we verify this condition for a 3D lattice 100x100x100, without obstacles and for different concentrations of enzyme and substrate. The values of the fractal parameter *h* in these conditions are given in table IV.

Table I. Values of fractal parameter *h* for a 3D lattice with different initial concentrations of S and E, without obstacles

Initial concentrations	*h*
[S]=0.1, [E]=0.01	0.024±0.005
[S]=0.2, [E]=0.01	0.046±0.002
[S]=0.2, [E]=0.02	0.033±0.002

The obtained values of the fractal parameter are in good accordance with the results obtained by Schnell and Turner [2]. Their values h=0.03 for [S]=0.1 and [E]=0.01 correspond to a smaller lattice (40x40x40) and reduced number of iterations (50). Using the same initial concentrations of E and S we have obtained h=0.024. This value is more accurate due to larger simulation, bigger lattice and higher number of iterations. Even though *h* values are slightly different from zero (due to auto crowding of substrate molecules) our simulation data are sufficiently accurate to illustrate that the algorithm offers results according with theoretical predictions.

B) The comparison of enzymatic kinetics in 2D and 3D crowded media

Simulation data show that in the presence of obstacles reaction kinetics is always fractal. Moreover, the degree of fractality is dependent on obstacle density being grater for simulations in 2D than in 3D. The explanation resides in the fact that in two dimensional media diffusion does not offer a perfect mixing of particles inducing thus the fractal kinetics.

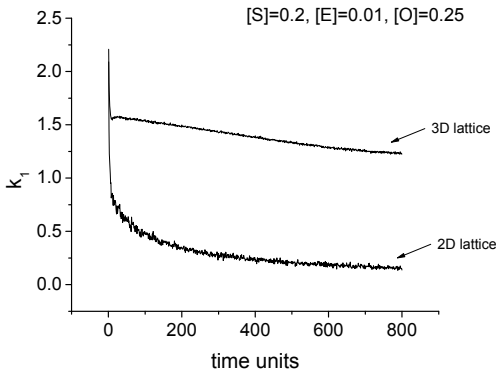

Figure 1. The comparison of temporal dependence of k_1 for two and three dimensional media

Figure 1 illustrates a higher degree of fractality for reactions occurring in 2D media than in 3D ones. The two curves correspond to simulations performed in 2D and 3D lattices in which identical concentration of reactants ([S]=0.2, [E]=0.01) and obstacle density ([O]=0.25) have been initially input.

Table II. Values of *h* and τ parameters for 2D and 3D lattices with mobile obstacles

lattice	*τ*	*h*
2D	63.85±5.3	0.270±0.003
3D	1096±186	0.083±0.001

The mobility of obstacles generates a reduced fractality of 3D media compared with that of 2D media, indicated by a lower value of *h* in table II, and the time after which the reactants perceive the macromolecular crowding is prolonged.

It is well known that rate coefficient in classical kinetics is only dependent by the relative diffusion coefficients of reactants, being independent of their initial concentration and time, too. Instead, in crowded media rate coefficient is influenced by the obstacle density and topological dimension of the medium due to their direct impact on the diffusion coefficients. For diffusion controlled bimolecular reactions the rate constant is proportional with diffusion coefficient $k_0 \sim D$ and D for media with $d \geq 2$ depends on particles concentration $D \sim 1 - c$ [16].

Even though our simulation data present small variations of k_0 for different initial concentrations of enzyme and substrate, Figure 2 indicates decreasing values of k_0 along with the rise of obstacle density. Also, this figure reveals the dependence of rate constant on the topological dimension of the medium.

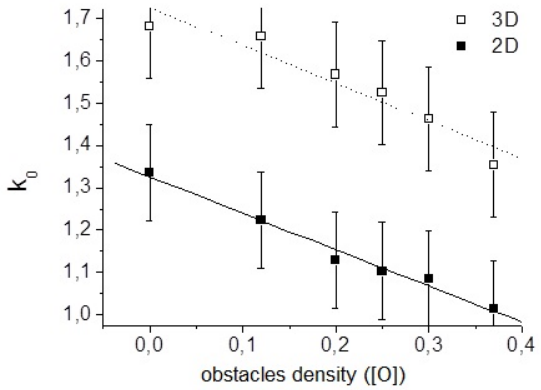

Figure 2. Medium values of the rate constant k_0 (with errors) versus obstacle density in 2D (■) and 3D (□) media. The lines correspond to linear fittings of data

This linear diminution of rate constant values along with the rise of obstacle density indicates the impact of crowding on reaction kinetics: a greater crowding corresponds to smaller diffusion coefficients. The higher values of k_0 in 3D media illustrate a better mixing of the particles than in 2D ones.

C) The effect of obstacle mobility on kinetics fractality

For this case we consider 3D lattices with identical obstacle density ([O]=0.25) but distinct degree of their mobility (0% and 100%, respectively) starting from the same initial concentration of the reactants: [S]=0.2, [E]=0.010. Thus, time dependence of rate coefficient k_1 for these two situations is presented in Figure 3.

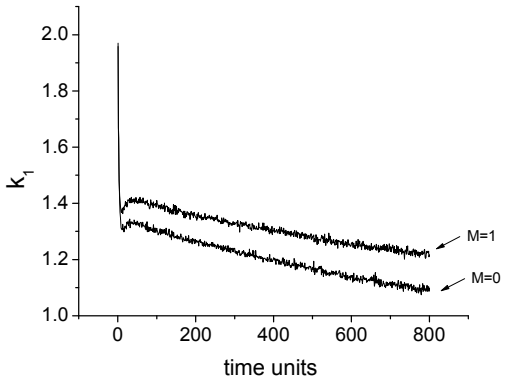

Figure 3. Temporal dependence of k_1 for different mobility of obstacles ([S]=0.2, [E]=0.01, [O]=0.25)

It can be seen that for mobile obstacles, reactants spread easily through the lattice conducting to a less fractal kinetics.

Table III. Values of *h* and *τ* parameters for a 3D lattice with different mobility of obstacles

Mobility	τ	h
0%	1338±231	0.961±0.136
100%	1342±240	0.707±0.103

The h values reflect the fractality of the media. When obstacles are mobile, reactants seem to travel a less crowded volume in order to meet and react, indicated by a lower h value. Instead, the obstacle mobility does not affect the time of transition from the classical to the fractal regime.

Obstacle mobility has only minor effect on rate constant value. It slowly increases from 1.395 to 1.477 with the rise of obstacle mobility being related to higher diffusion coefficients of reactants.

CONCLUSIONS

For systems without obstacles, particles are mixed more efficiently in 3D lattices than in 2D. As a result, fractal parameters values are very small and the kinetics tends to be classical. These results are in good accordance with other simulation data reported by Berry

(2002) and Schnell and Turner, respectively [2].

Instead, when obstacles are present kinetics is fractal in both 2D and 3D media. The degree of fractality is always smaller in 3D than in 2D due to a better mixing of the reactants. Also, it increases with the rise of obstacle density (crowding growth) and decreases with the rise of their mobility (increase of reactants mixing).

Molecular crowding brings negative effects on reaction kinetics. Reduction of diffusion coefficients of reactants contributes to diminution of collision rate between them and thus reduces the rate coefficient values.

All data presented in this study together with those published in specific literature indicate that both computational and experimental approaches of kinetics of enzymatic reactions must take into account the effects brought by molecular crowding.

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