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Monte Carlo simulations of enzymatic reactions in crowded media. Effect of the enzyme-obstacle relative size

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ABSTRACT

We perform Monte Carlo simulations in three-dimensional (3D) lattice in order to study diffusion-controlled and mixed activation-diffusion reactions following an irreversible Michaelis–Menten scheme in crowded media. The simulation data reveal the rate coefficient dependence on time for diffusion-controlled bimolecular reactions developing in three-dimensional media with obstacles, as predicted by fractal kinetics approach. For the cases of mixed activation-diffusion reactions, the fractality of the reaction decreases as the activation control increases. We propose a modified form of the Zipf–Mandelbrot equation to describe the time dependence of the rate coefficient, $k(t) = k_0(1 + t/\tau)^{-h}$. This equation provides a good description of the fractal regime and it may be split into two terms: one that corresponds to the initial rate constant (k_0) and the other one correlated with the kinetics fractality. Additionally, the proposed equation contains and links two limit expressions corresponding to short and large periods of time: $k_1 = k_0$ (for $t \ll \tau$) that relates to classical kinetics and the well-known Kopelman's equation $k \sim t^{-h}$ (for $t \gg \tau$) associated to fractal kinetics. The τ parameter has the meaning of a crossover time between these two limiting behaviours. The value of k_0 is mainly dependent on the excluded volume and the enzyme-obstacle relative size. This dependence can be explained in terms of the radius of an average confined volume that every enzyme molecule feels, and correlates very well with the crossover length obtained in previous studies of enzyme diffusion in crowding media.

1. Introduction

Many functions of living cells involve complex biochemical reactions of which rates must be as fast as possible to allow a wide range of processes to take place. To study biochemical reactions one must take into account that cellular media are not homogenous but highly compartmented being characterized by a high total macromolecular content known as macromolecular crowding [1–3]. Macromolecular crowding influences the thermodynamics of the cell by volume exclusion effects [1–3]. It also affects the diffusion processes by reducing the diffusion coefficient of the macromolecules [4–20]. In this way, diffusive processes leading to the necessary encounter of reactants determine the rates of biochemical reactions. However, after the pioneering work of Laurent in 1971 [21], quite a few studies have explored the effects of crowding on enzyme catalysis, even *in vitro* [22–39].

Schnell and Turner [40] excellently reviewed a few non-classical approaches regarding biochemical reactions developing in crowded media. They are mainly divided into deterministic approaches, particularly comprising fractal kinetic approaches [41–42] and kinetics based on fractional reaction orders [43–46], and stochastic ones [47]. However, there are mathematical connections between deterministic and stochastic models. The conversion between these models has been illustrated for large-scale genetic regulatory networks [48] and for Michaelis–Menten enzyme kinetics and stochastic focusing [49]. The deterministic models proved to be widely applied on the analysis of enzymatic reactions developing in crowded media. Among them, the fractal-like kinetics is the one considered in the present study. The fractal-like kinetics

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assumes that the rate coefficient describing diffusion controlled chemical reactions is time dependent, for large periods of time, taking the form [41–42]:

$$k(t) = k_0' t^{-h}; \quad 0 \leqslant h \leqslant 1 \tag{1}$$

This formalism brakes down for $t \to 0$ and $h > 0$ because in this situation $k(t) \to \infty$. A solution has been proposed by Schnell and Turner [40] and they consider a modified fractal-like kinetics with the rate coefficient following a temporal Zipf–Mandelbrot distribution:

$$k(t) = \frac{k_0''}{(\tau + t)^h}; \quad 0 \leqslant h \leqslant 1 \tag{2}$$

In both Eqs. (1) and (2), k_0' and k_0'' are constant even though not related to any rate constant as in classical kinetics and h, called the fractal parameter, depends on the topological dimensionality of the medium in which reaction occurs. The τ parameter in Eq. (2) is a positive constant and its physical meaning is the time after which the reaction "feels" the crowding effects [40].

Recently, Bajzer et al. [44,45] and Neff et al. [46] have proven that Schnell and Turner model applied on bimolecular reactions predicted an unlikely asymptotic concentration of the product. This approach is valid for diffusion-limited reactions and we used it in our investigation assuming that the corresponding time period of our simulations does not reach the asymptotic region. Moreover, for enzymatic reactions in crowded media, the diffusion-limited case is not the most usual one [16,33,38, and references quoted therein]. There are also cases of mixed activation-diffusion control, e.g., the reduction of Pyruvate by NADH catalyzed by Lactate Dehydrogenase induced by Balcells et al. [39]. For these last cases, the asymptotic concentration of the product is reached at longer periods of time than for diffusion-limited reaction cases and, thus, the time period used in simulations can be larger.

To perform *in vivo* experiments of biochemical reaction dynamics in crowded media is a difficult task, therefore computer simulation is an excellent alternative. There are numerous published papers with regard to simulations of diffusion processes [4–7,10–12,17,19–20] and chemical reactions in crowded media [40–47,50–56]. Macromolecular crowding can lead to anomalous diffusion more likely when low dimension environments are considered especially up to percolation threshold [41]. In such cases, diffusion is strongly influenced by crowders concentration, size and mobility. It has been illustrated that diffusion becomes more anomalous when higher concentration of obstacles is present but less anomalous for mobile and big obstacles [10–12,14,17–20]. Inside living cells, macromolecular crowding does not always reach the percolation limit. However, when large macromolecules diffuse inside the cell their movement is greatly impaired [8,15]. Consequently, anomalous diffusion becomes perceptible and significant giving rise to rate-limited chemical reactions [16].

Berry [52] studied a native Michaelis–Menten reaction scheme using a Monte Carlo simulation on two-dimensional lattice with cyclic boundary conditions. He used immobile obstacles varying their densities from zero to the percolation threshold. His study showed that the reaction kinetics was of fractal type as a result of low-dimensional media and macromolecular crowding. The kinetics fractal characteristics intensified with obstacles density and substrate concentration, the two contributions being mainly additive.

Schnell and Turner [40] used Berry's algorithm and implemented it in a two-dimensional lattice with cyclic boundary conditions using the same parameters [52]. Apart from confirming the clear decay of the rate coefficient k_1 over time, they also revealed that the rate coefficient behaviour at $t \to 0$ was better described by the Zipf–Mandelbrot distribution than the Kopelman's law. They extended the simulation to a three-dimensional lattice without obstacles for which case the rate coefficient did not show an apparent dependence on time, being thus in accordance with classical kinetics.

Isvoran et al. [57] analysed several computational aspects of the implementation of Berry's algorithm in two-dimensional media with obstacles. Particularly, different initial distributions of obstacles and reactants molecules were considered and also the effect brought by eight nearest neighbours of every particle in the lattice instead of four. Moreover, Isvoran et al. [58] compared different equations proposed in the literature in order to describe the rate coefficient time dependence.

Agrawal et al. [59] studied the effect of macromolecular crowding on Michaelis–Menten enzymatic reaction occurring in 2D media. They used a Monte Carlo algorithm based on substrate molecules random walk in a percolation cluster. The substrate diffusion length and the reaction rate decrease as the fractional volume occupancy of the crowding agent increases.

Intracellular structures have been explicitly modelled using immobile/mobile obstacles in 2D environments for the first time by Grima and Schnell [55]. They compared different geometries of the simulation grid with an off-lattice Brownian Dynamics, revealing qualitative and quantitative differences as the obstacle concentration increased. Moreover, Grima [56] extended the off-lattice simulation study to the case of activation control in crowded media, showing that crowding induces a reduction of the noise of intracellular biochemical reactions. Recently, Klann et al. [60] have developed an off-lattice continuous space simulation method, in which all sub-cellular structures are modelled explicitly as static obstacles. They noticed a reduced reaction rate in presence of obstacles. They also identified some factors causing the diminution of the reaction rate: the reduced mobility of the reactants and molecular crowding determining a reduced accessibility of the molecules.

Moreover, there are some computational and theoretical papers emphasising the effect of diffusion on enzyme kinetics in cellular environment [61–65, and references therein]. These studies provide several expressions concerning the time-dependence of reactants diffusion coefficients and the effects of the excluded volume resulting in time-dependent rate coefficients.

In recent years, the effects of crowding on enzyme catalysis have been explored by different experimental works, excellently depicted by Zhou et al. [16] and Noris and Malys [30]. Some of the studies present distinct effects produced by various crowding agents on the enzymes kinetics [26,33,38–39,66]. These effects are given by different experimental conditions considering the type, shape, size and excluded volume of the used crowders. Anyway, most of the investigations indicate that the effect of excluded volume due to the presence of crowding agent is the major player in modulating enzymatic behavior.

However, the dependence of the kinetic parameters of a typical Michaelis–Menten enzymatic mechanism on the enzyme-crowder relative size and their particular shape is not yet well understood.

As a result, the present study aims at extending Berry's algorithm in order to examine a more nature-like environment, namely to analyse an irreversible Michaelis–Menten enzymatic reaction progressing in 3D crowded media, not only for diffusion-limited reaction cases but also for mixed activation-diffusion control ones. In detail, the work attempts to express the time-dependence of the bimolecular rate coefficients in terms of excluded volume and enzyme-obstacle relative size. A modified form of Zipf–Mandelbrot equation, previously proposed by our research team [67], is used to describe the time dependence of the rate coefficient $k(t) = k_0(1 + t/\tau)^{-h}$. Recently, this kind of equation has also been taken into account by Bajzer et al. [46]. They also generalize different approximations used in the literature in order to understand which