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DESCRIPTION OF THE PRECONDITIONING EFFECT IN BIOLOGY BY MEANS OF FRACTAL-LIKE ENZYME KINETICS OF INTRACELLULAR REACTIONS

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Preconditioning is the process of subjecting a cell or a tissue to a stimulus, with the result that the cell or the tissue becomes more tolerant to a second insult. The signalling machinery involved in preconditioning can be seen only as an ordered succession of enzymatic reactions. The fractal organization of the cytoplasm seems to be at present well proved. In classical chemical kinetics the rate constant of reaction is independent of time. However, recent studies have shown that the rate constant is time-dependent in many reactions. The main change is that the rate coefficient takes the following form: $k(t) \sim k\, t^{-h}$, $0 \le h \le 1$. The kinetics of an enzymatic reaction was considered in a fractal-like environment. A given time after the first stimulus, one returns to the initial conditions except that the change of the environment (the fractal dimension) which is conserved. At the second challenge the reaction rate is clearly less than at the first one. We conclude that inside cells the reaction kinetics is very well described by fractal-like kinetics and that this type of enzyme kinetics may explain at least in part the preconditioning effect.

INTRODUCTION

Preconditioning represents a process of subjecting a cell or a tissue to a stimulus, with the result that the cell or tissue becomes more tolerant to a subsequent insult.[1] The cellular basis of the mechanism underlying preconditioning is not fully understood. New insights in this field may open future perspectives in the management of some severe conditions as myocardial infarction, stroke or sepsis. Several hypotheses have been proposed to answer this question, but no satisfactory explanation with an unifying concept has been provided.[2]

Intracellular environments are characterised by a high total macromolecular content, known as macromolecular crowding. It is widely accepted by biologists that cytoplasm cannot be considered as a simple Newtonian fluid.[3] There is evidence for the hypothesis that the solid phase of cytoplasm serves as a support for the immobilization of enzymes and as a global framework for the functional integration of the whole cell.[4] Diffusion in cytoplasm can be accounted for by modelling cytoplasm as a network of filaments with a crowded fluid phase.[4] The intracellular environment can be seen as a structured, organized, macromolecular assembly with a complex, fractal architecture. A fractal view of the cytoplasm of living cells follows the 'structured' view and introduces new feasible behavioural possibilities.[3] However, it appears obvious in the light of these recent data that the non-ideality of the cell interior may necessitate an alternate view of cellular biochemistry.[4]

The cell can be understood as a complex network of catalyzed reactions[5] and it is obvious that enzymes are the center of every biochemical process. Therefore, every attempt to understand this complex process may take into account the

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enzyme kinetics. The law of mass action considers chemical reactions to be continuous and deterministic under convective or diffusive stirring. These are evidently simplifications, as it is well understood that chemical reactions involve discrete, random collisions between individual molecules. As we consider smaller chemical systems and intracellular environments, the validity of a continuous approach becomes ever more tenuous. As such, the adequacy of the law of mass action has been questioned for describing intracellular reactions.[6]

The kinetics of catalytic reactions in fractal media is well studied. As is known, the rate constant of reaction is independent of time in classical chemical kinetics. But, recent studies have shown that the rate constant is time-dependent in many reactions.[7] The geometric heterogeneity results in fractal-like kinetics and is relevant to experiments in porous membranes, films, and polymeric glasses.[7]

Classical reaction kinetics has been found to be unsatisfactory when the reactants are spatially constrained on the microscopic level by walls.[7] In classical kinetics, we do not expect the rate constant to have any time dependence. However, experimental studies on the reaction kinetics of excitons in molecular macroclusters (inside crystalline isotopic alloys) that were prepared as fractals yielded very anomalous results in which rate constants depend on time.[8]

The reaction environments of in vivo conditions are characterized by macromolecular crowding, small volumes, spatial non-uniformity and significant physical structure. The obtained theoretical and experimental results demonstrate that diffusion limited elementary reaction are highly affected by the spatial dimension in which they occurs.[6] The law of mass action breaks down in spatially heterogeneous environments with obstacles like cells.

When minimal obstructions to diffusion are present, the rate constant k remains constant over time. In the presence of significant obstruction to diffusion, log k decays linearly on a logarithmic timescale.[8]

The rate constant with a maximal level of obstruction to diffusion has the form:

$$\log k = -h \log t + \log k_0 \qquad (1)$$

where $k_0 \cong k\,(1)$ and h is an arbitrary constant. Equation (1) can be re-written as: [8]

$$k(t) = k_0 t^{-h}; 0 \le h \le 1\,(t \ge 1) \qquad (2)$$

This time-dependent form is applicable for rate constants of diffusion controlled reactions *i.e.* those of second order or higher. First order reactions remain time independent in *k* because the reaction of a single molecule is unaffected by constraints on diffusion and mixing. The constant *h* is a measure of the dimensionality of the system. In a three dimensional homogeneous environments $h = 0$ and *k* is a constant. In diffusion limited reactions that occur in fractal spaces, $h > 0$.[8] These include reactions in heterogeneous systems, in different phases, enzymatic or membrane reactions. That is why *h* is called "fractal parameter".[8] The purpose of this study was to examine the hypothesis that a fractal-like kinetics may explain, at least in part, the preconditioning effect in biology.

Enzymatic reactions in a fractal environment and preconditioning: a mathematical model

We consider the kinetics of an enzymatic reaction (firstly without the quasi steady state assumption and afterwards with the steady state assumption (Michaelis-Menten) in a fractal-like environment (this is expressed by letting k_1 be a function of time but preserving the condition of enzyme conservation). The model is intended to reflect a progressive "change" of the environment. After a given time one returns to the initial conditions (by eliminating the product) except that the change of the environment is conserved (in what follows this is also called the "second challenge"). A kind of "preconditioning" is observed. The stimuli are not present in an explicit way in this model but a more complex model, taking them into account, will be considered in a forthcoming paper.

The general (non fractal) enzymatic reaction can be written as:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} E + P \qquad (3)$$

The usual notations are: *S* is the substrate, *E* is the enzyme, *C* is the complex (substrate-enzyme), *P* is the product and k_1, k_{-1}, k_2 the kinetic constants.

A. The general system

The previous reactions are described by the system of differential equations:

$$\frac{d[S]}{dt} = -k_1[S][E] + k_{-1}[C]$$

$$\frac{d[E]}{dt} = -k_1[S][E] + (k_{-1} + k_2)[C] \qquad (4)$$

$$\frac{d[C]}{dt} = k_1[S][E] - (k_{-1} + k_2)[C]$$

$$\frac{d[P]}{dt} = k_2[C]$$

with the values $[S_0], [E_0], [C_0], [P_0]$ at $t = 0$. The usual hypothesis are: at $t_0 = 0, [C_0] = [P_0] \approx 0$ By adding the second and the third equations, one obtains the enzyme conservation:

$$E + C = E_0 \text{ (constant)} \qquad (5)$$

We mention that in the above computations we did not use any special assumption.

B. Fractal-like kinetics

We let the coefficient k_1 be time-dependent, *i.e.* $k_1(t)$. According to Kopelman[7] the fractal-like kinetics is characterized by $k_1(t) = k_1 t^{-h}, h \in (0,1)$, k_1 being a constant. We do not consider, in this model, that k_{-1} and k_2 change in time. The above system becomes:

$$\frac{d[S]}{dt} = -k_1 t^{-h}[S][E] + k_{-1}[C]$$

$$\frac{d[E]}{dt} = -k_1 t^{-h}[S][E] + (k_{-1} + k_2)[C] \qquad (6)$$

$$\frac{d[C]}{dt} = k_1 t^{-h}[S][E] - (k_{-1} + k_2)[C]$$

$$\frac{d[P]}{dt} = k_2[C]$$

C. The quasi-steady state assumption

The quasi-steady state assumption is written as $\frac{d[C]}{dt} = 0$ which leads to the usual Michaelis-Menten equation:

$$\frac{d[S]}{dt} = -\frac{V_m[S]}{[S] + K_M} \qquad (7)$$

with $V_m =$ the maximal velocity and $K_M = \frac{k_{-1} + k_2}{k_1}$ = the Michaelis-Menten constant.

D. Fractal-like kinetics and the Michaelis-Menten equation

As before (see case B), for $k_1(t) = k_1 t^{-h}$, the quasi steady state assumption leads to the equation:

$$\frac{d[S]}{dt} = -\frac{V_m[S]}{[S] + K_M(t)} \qquad (8)$$

with $K_M(t) = \frac{k_{-1} + k_2}{k_1 t^{-h}}$, and:

$$\frac{d[S]}{dt} = -\frac{V_m[S]}{[S] + (k_{-1} + k_2)k_1^{-1} t^h} \qquad (9)$$

RESULTS AND DISCUSSION

1. Simulations

Computer simulations, performed with the MatLab 6.5 software, delivered the concentration variation of important species S, E, C and P. For all the simulations we used the values: $k_1 = k_{-1} = 10$, $k_2 = 1$, the initial values $[S_0] = 1, [E_0] = 0.1, [C_0] = [P_0] = 0$, and the time intervals [0; 60] and [60; 120], respectively (according to Rao [9]). The cases 1-5 refer to the system (A or B), while the cases 6-10 refer to Michaelis-Menten Equation (C and D).

2. Results

The results obtained by simulations using the above mentioned values are presented in Figs. 1 – 6. Fig. 1 represents the simulation of the original system (A). Fig. 4 represents the simulation of the Michaelis-Menten equation (C). One can observe that the QSSA is valid.
Preconditioning: Case 2 and Case 3 (for the system, *h=0.8*), Fig. 2.
Preconditioning: Case 4 and Case 5 (for the system, *h=0.3*), Fig. 3.
Preconditioning: Case 7 and Case 8 (for the Michaelis-Menten Equation, *h=0.8*), Fig. 5.
Preconditioning: Case 9 and Case 10 (for the Michaelis-Menten Equation, *h=0.3*), Fig. 6.

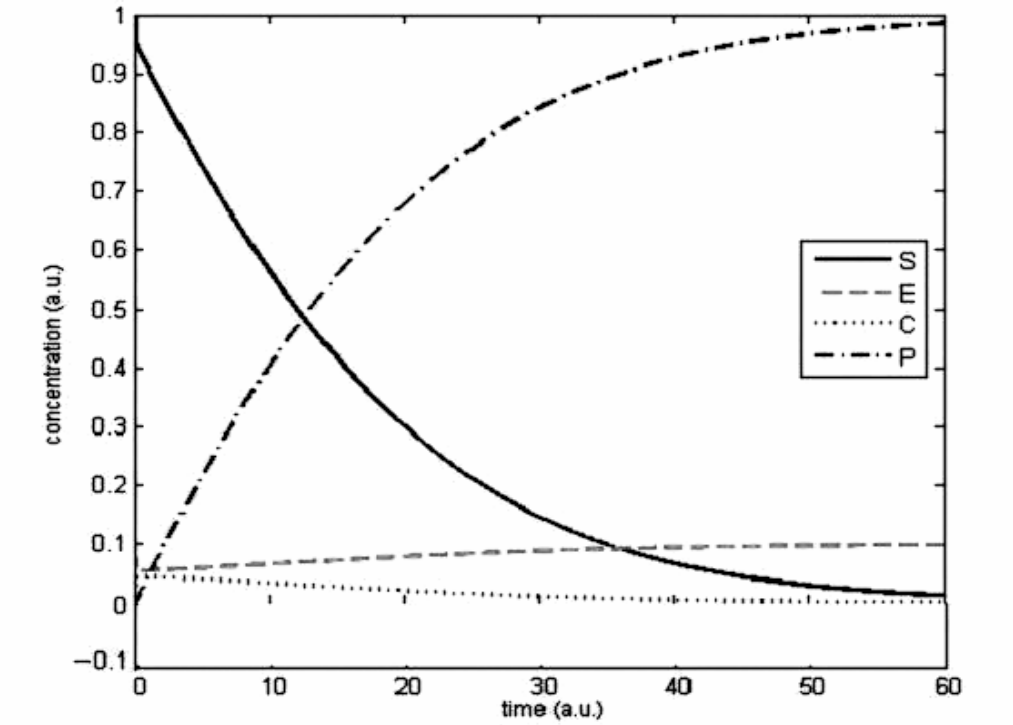

Fig. 1 – The simulation of the original system (A).

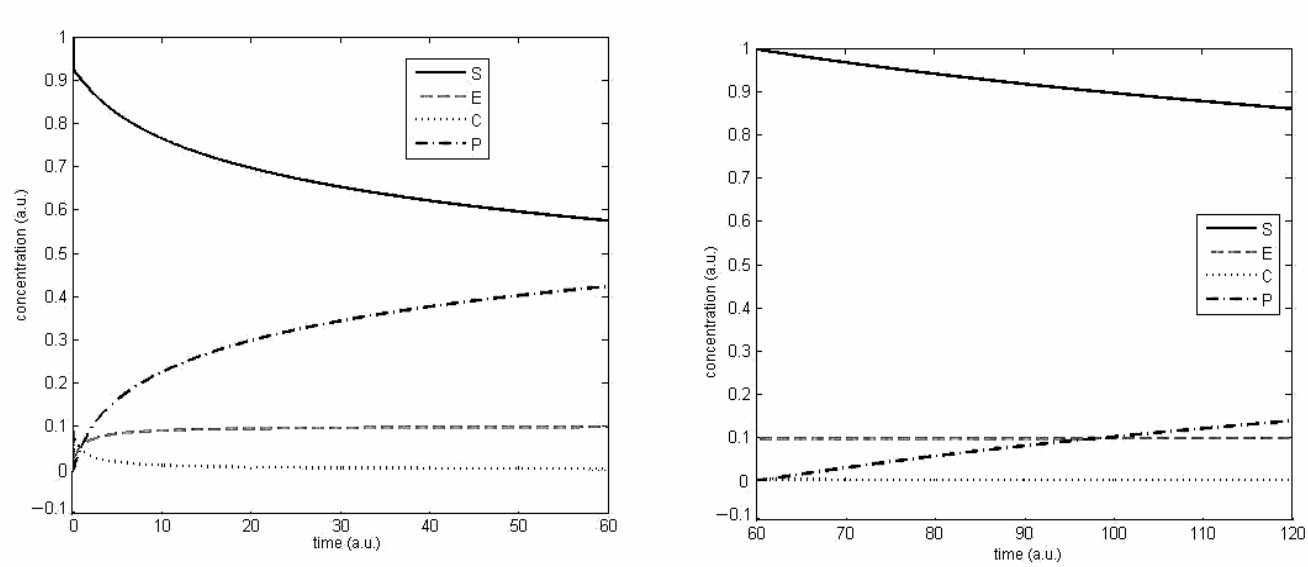

Fig. 2 – Fractal-like kinetics simulation in the system (B) for h=0.8. We considered a second stimulus at the moment t=60 (taken as origin).

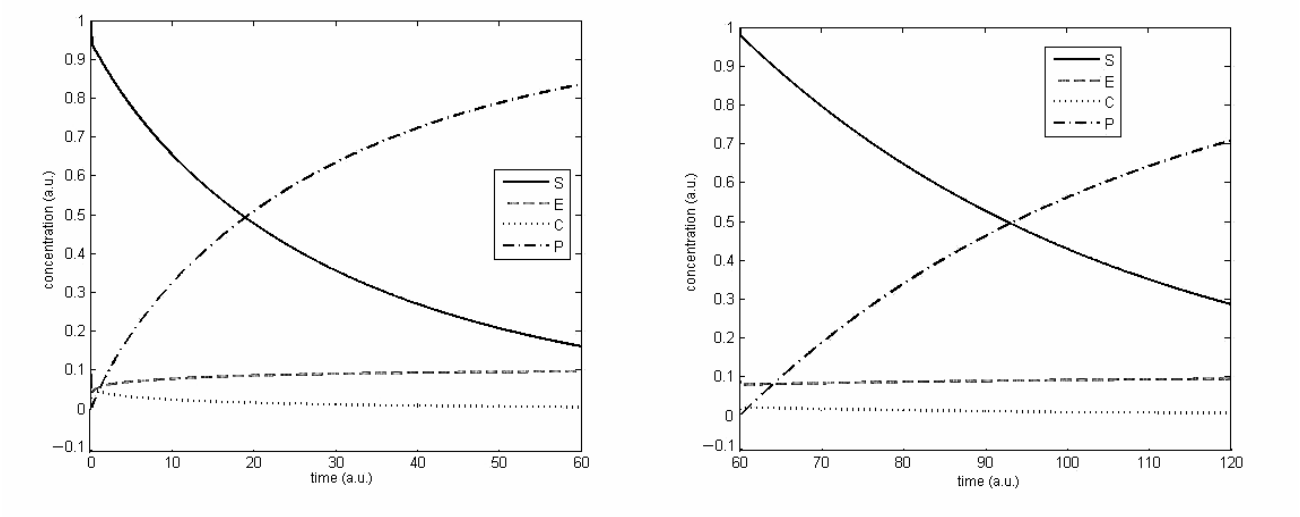

Fig. 3 – Fractal-like kinetics simulation in the system (B) for h=0.3. We considered a second stimulus at the moment t=60 (taken as origin).

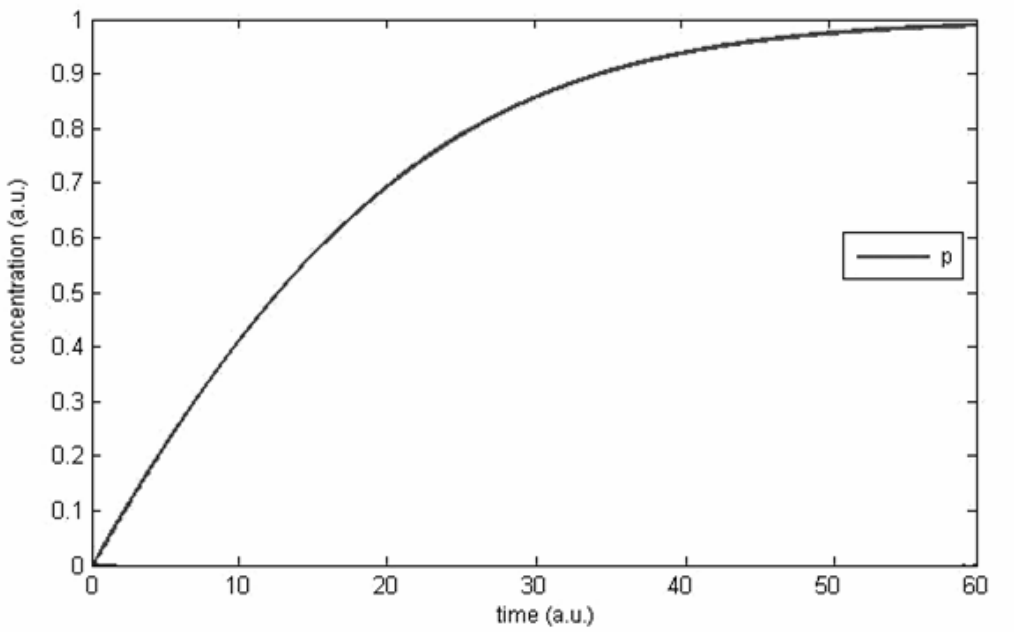

Fig. 4 – The simulation of the Michaelis-Menten equation (C).

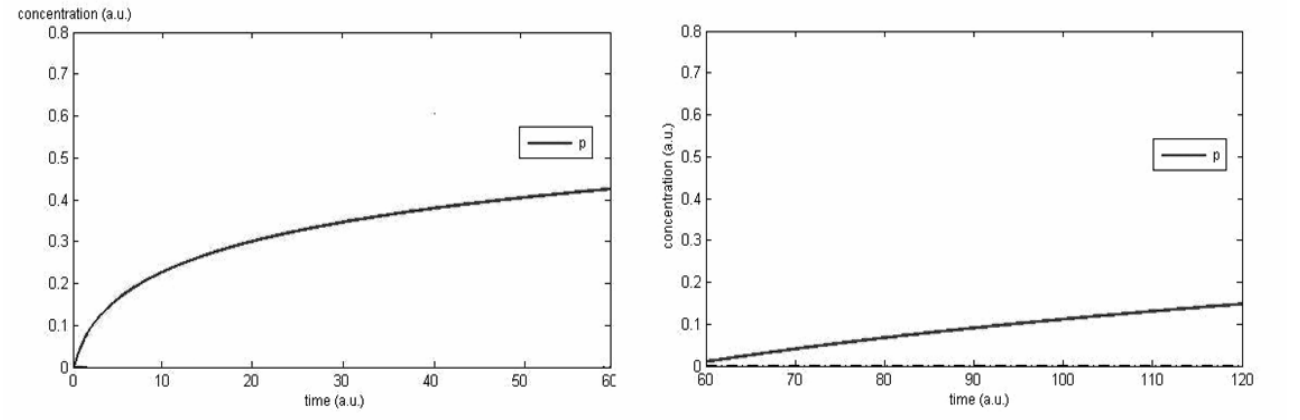

Fig. 5 – Fractal-like kinetics in Michaelis-Menten equation (D) with h=0.8.We considered a second challenge (stimulus) at the moment t=60 (taken as origin).

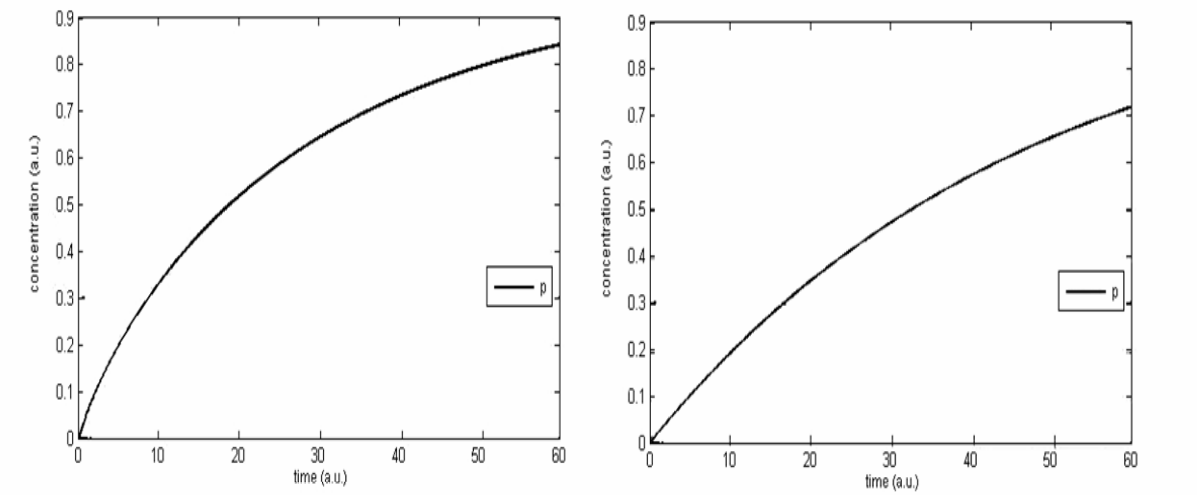

Fig. 6 – Fractal-like kinetics in Michaelis-Menten equation (D) with h=0.3. We considered a second challenge (stimulus) at the moment t=60 (taken as origin).

3. Discussion

There are differences between the fractal-like kinetics and the classical case (Fig. 1 with Fig. 2). It seems that QSSA for fractal kinetics still gives a good approximation of the solution (Fig. 2 compared with Fig. 5).

A given time after the first stimulus one returns to the initial conditions (by the elimination of the product) with the exception that the change of the environment (the fractal dimension) is conserved. At the second challenge the rate is clearly less than at the first one (the product increases slower).

In our scenarios two different values of h were considered*: h = 0.8* and *h = 0.3*. As expected, the preconditioning is "effective" for greater values of h (compare *h=0.3* and *h=0.8*).

The idea behind these simulations is that we considered possible that *h* itself be a function of time. This point of view will be discussed in a forthcoming work. A particular case of preconditioning, namely endotoxin tolerance, was studied in previous papers.[10-15] In building the mathematical model the idea of a brake of proinflammatory signaling machinery was used.[10,11,16]

Kopelman showed that the diffusion-limited reaction rate for the elementary reaction A+A→ product in a fractal medium is proportional to t^{-h} $[A]^2$ for batch conditions. The increase in *h* with decreasing dimensionality reflects deviations from the classical law of mass action. These deviations are the result of dimensional or topological constraints in which convective or diffusive stirring is inefficient.[17,18] The fractal-like kinetics model works well in low-dimensional non-fractal systems.[19]

Another approach for heterogeneous catalysis is the "power-low approximation".[20] This topic will be discussed in a future paper.

The reaction kinetics seems to dependent on the geometry and the size of the intracellular surfaces on which the reactions occur.[17,18] Thus, we believe there is strong preliminary evidence allowing us to advance this hypothesis demonstrating the importance of fractal-like enzyme kinetics in the preconditioning effect in biology.

Preconditioning by ischemic tolerance was first identified in the heart by Murry *et al*[21] and was subsequently found to occur in the brain and a variety of organs including liver, intestine, kidney, and lung.[22] Subsequently, thermal, anaesthetic and pharmacological preconditioning have shown equally impressive results.[23] A repeated treatment with endotoxin can lead to desensitization of subsequent pro-inflammatory cytokine responses. This phenomenon, known as "endotoxin tolerance", is a well-established paradigm of preconditioning. Preconditioning is emerging as a simple, safe and highly effective means of attenuating local and systemic effects of medical and surgical insult. Its enormous potential in neurosurgery, cardiac surgery, orthopaedics and sepsis[10,11] has not yet been harnessed and ongoing work will continue to bring it to the fore.[23] The mechanism of preconditioning is with certitude a complex one. Multiple mediators, negative feedback loops as micro RNAs,[12,13] mitochondrion and other players may be involved.

In all these biological mechanisms preconditioning sets off the release of triggers, which in turn cause an intracellular cascade to produce end-effectors. Finally, these end-effectors are thought

to limit the injury after a second challenge. However, the triggers are received by the cell and an intracellular enzyme cascade then changes the phenotype of the cell. This enzyme cascade is difficult to understand but must obey the general enzyme kinetics laws. Our hypothesis is that the fractal organization of the intracellular environment imposes diffusion limits followed by a fractal-like enzyme kinetics.

CONCLUSIONS

As a result of our simulations we conclude that inside cells the reaction kinetics is very well described by fractal-like kinetics. This type of enzyme kinetics may explain at least in part the preconditioning effect.

This idea offers a theoretical support for modulation of the enzymatic activity of the cell by changing the fractal dimension of the cytoskeleton.

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