

Fractal-like kinetics, a possible link between preconditioning and sepsis immunodepression. On the chemical basis of innate immunity

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Rezumat

Cinetica fractală, o posibilă legătură între efectul de precondiționare și imunodepresia pacientului septic. Despre bazele chimice ale imunității innăscute

Autorii au lansat într-un articol recent ipoteza că efectul de precondiționare în biologie ar putea fi explicat de cinetica fractală a reacțiilor enzimactice intracelulare (Vasilescu C, Olteanu M, Flondor P, *Revue Roumaine de Chimie*. 2011;56(7): 751-7). Modelul bazat pe cinetica fractală descrie bine cinetica reacțiilor intracelulare. În articolul de față sunt discutate unele implicații clinice ale acestui model. Toleranța la endotoxină constituie un caz particular de precondiționare și prezintă numeroase similarități cu depresia imună întâlnită la unii dintre pacienții septici. Dacă *fractal-like kinetics* joacă un rol în fiziopatologia sepsisului, această idee poate constitui baza teoretică a unor metode terapeutice de imunomodulare bazate pe modificare dimensiunii fractale a citoscheletului.

Cuvinte cheie: cinetica fractală, precondiționare, toleranță la endotoxină, sepsis, imunitate innăscută, fractali

Abstract

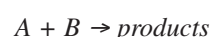
In a recent paper the authors hypothesized that the so called fractal-like enzyme kinetics of intracellular reactions may explain the preconditioning effect in biology (Vasilescu C, Olteanu M, Flondor P, *Revue Roumaine de Chimie*. 2011; 56(7): 751-7). Inside cells the reaction kinetics is very well described by fractal-like kinetics. In the present work some clinical implications of this model are analyzed. Endotoxin tolerance is a particular case of preconditioning and shows similarities with the immunodepression seen in some sepsis patients. This idea offers a theoretical support for modulation of the enzymatic activity of the cell by changing the fractal dimension of the cytoskeleton.

Key words: fractal-like kinetics, preconditioning, endotoxin tolerance, sepsis, innate immunity, fractals

What is fractal-like enzyme kinetics?

The various intracellular signal transduction pathways can be seen as chains of enzymatic reactions. The kinetics of these reactions obeys the well-known Michaelis-Menten equations (1). According to these laws the bimolecular reaction rate of the product is proportional to concentrations of the reactants

The Michaelis-Menten equation describes the relationship between the rate of substrate conversion by an enzyme to the concentration of the substrate



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All of the concentration dependence of the reaction can be expressed either as

$$\text{Rate} = K[A][B]$$

where $[A]$ is the reactant concentration (or density) of A and K is the rate constant. Note that K is independent of time (2).

This classical reaction kinetics has been found to be unsatisfactory when the reactants are spatially constrained on the microscopic level. Kopelman was the first to publish a model of heterogeneous reaction kinetics have dramatic consequences, such as fractal orders for elementary reactions, self-ordering and self-unmixing of reactants, and rate coefficients with temporal "memories". He coined the term "fractal-like kinetics" (2,3). Among the practical examples of "fractal-like kinetics" are chemical reactions in pores of membranes, excitation trapping in molecular aggregates, exciton fusion in composite materials, and charge recombination in colloids and clouds. To emphasize this time dependence, K is replaced by k

$$k(t) = kt^{-h}, 0 \leq h \leq 1, t \geq 1$$

These kinetic laws are established considering experiments performed in vitro, in the so-called "ideal conditions": homogenous (well stirred) reaction medium, at high dilutions and in a practically infinite reaction volume.

According to (3) "for diffusion-limited reactions that occur in fractal spaces, theory (and simulations) give $h > 0$ and hence a time-dependent k "

This time-dependent form is applicable for rate constants of reactions in fractal media and in media with geometrical constraints as the macromolecular crowding of intracellular environment.

In a three-dimensional homogeneous environments $h = 0$ and k is a constant. In diffusion limited reactions that occur in fractal spaces, $h > 0$ (3). These include reactions in heterogeneous systems, in different phases, enzymatic or membrane reactions. That is why h is called "fractal parameter" (3,4).

The increase in h with decreasing dimensionality reflects deviations from the classical law of mass action.

Most of the reactions are taking place inside the cell. Heterogeneity, macromolecular crowding and compartmentalization characterize the morphology of the intracellular environment. This organization has profound implications on the diffusion of the reactants and therefore on the reaction rate. (Fig. 1, Fig. 2) However, the intracellular environment (in vivo) is certainly a non-ideal one, far from the conditions described above.

The fractal organization of the cytoplasm

Under the term fractal coined by Mandelbrot (5), different patterns are grouped which follow a geometry previously conceived as aberrant because they are undescrivable from either a mathematical or geometric viewpoints. The ground plan of living cells can be described as fractals (6). A fractal view of the cytoplasm of living cells follows the 'structured' view but introduces new feasible behavioral possibilities (6-9). The catalytic properties of enzymes at the cellular level are expected to be influenced by the fractal nature of the cytoplasm. Chemical reactions exhibit a fractal like kinetics when they are diffusion limited, dimensionally restricted or occur on fractal surfaces,

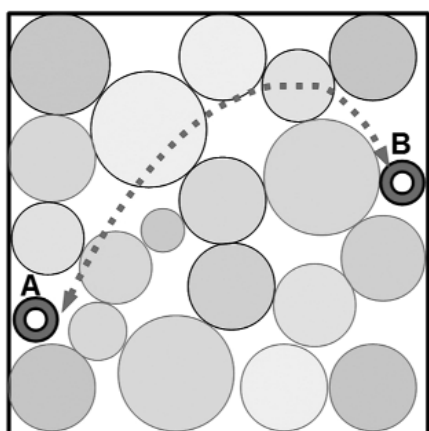


Figure 1. Macromolecular crowding, 2-D representation. "A" and "B" are single molecules of the two reactants. The circles represent macromolecules inside the cell. The collision of the molecules is impossible in this 2-D representation. So, the chemical reaction do not take place in this particular case

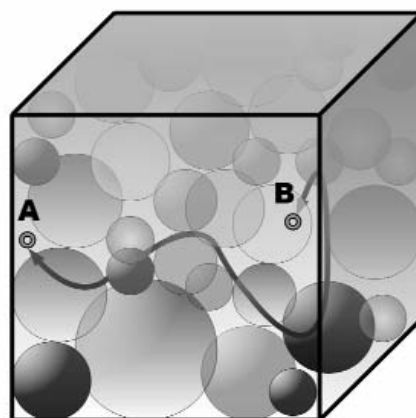


Figure 2. Macromolecular crowding, 3-D Representation. "A" and "B" are single molecules of the two reactants. The spheres represent macromolecules inside the cell. Its easy to observe that in 3-D, even at a high level of intracellular macromolecular crowding, a way between A and B remains open. So, the chemical reaction between A and B is still possible

conditions that reflect more accurately than homogeneity the natural context within living cells (1). So, the Michaelis-Menten equations are no longer valuable for the intracellular-reaction.

Intracellular medium is packed with small solutes, macromolecules, membranes and cytoskeletal proteins. The kinetics and thermo-dynamics of macromolecular processes and biochemical reactions taking place *in vivo* are known to be affected by such environments, characterized by macromolecular crowding (10). The complex structured and crowded intracellular conditions have a tremendous impact on intracellular reactions. Accordingly, the *in vivo* rate constants or even the structure of the kinetic rate expression can significantly differ from those obtained in *in vitro* assays. First of all, the crowded conditions squeeze all molecules closer together, which favors the formation of more compact complexes (11). Diffusion of proteins *in vivo* is significantly reduced compared to dilute conditions. According to Dlugosz and Trylska in the cytoplasm of eukaryotic cells, diffusion of both large and small molecules is slowed down three to four times (10).

What is preconditioning?

According to Riviere et al (2009) (12) preconditioning is a phenomenon in which second inflammatory stimulation (with either the same or different pro-inflammatory agent or stress) leads to one of three possible outcomes with regard to a single pro-inflammatory stimulus:

1. No difference, i.e. both the first and second responses are of the same magnitude;
2. Priming, in which the second response is greater than the first; or
3. Desensitization (tolerance), in which the second response is lower than the first.

Repeated treatment with Gram-negative bacterial lipopolysaccharide (LPS) is a well established paradigm of preconditioning.

As already shown, during infection, monocytes are one of

the main actors of innate immunity. Monocytes from patients with sepsis lose their ability to mount an inflammatory response after stimulation by LPS, a behavior showing striking similarities to the well known phenomenon of endotoxin tolerance (13-15). Endotoxin tolerance is defined as a diminished capacity to respond to a lipopolysaccharide challenge after a first exposure to this stimulus. In septic patients the proinflammatory reaction is often followed by an anti-inflammatory response resulting in an immunoparalytic state (16,17).

In endotoxin tolerance a cell develops reduced endotoxin responsiveness following repeated exposure to LPS. Pro-inflammatory cytokine secretion, especially TNF- α , is markedly diminished in endotoxin-tolerant animals and humans (18,19). (Fig. 3, Fig. 4) Common molecular mechanisms may underlie the hyporesponsiveness to LPS exhibited by monocytes from patients with sepsis and endotoxin-tolerant cells (20). The clinical significance of hyporesponsiveness to LPS in sepsis is still under debate. Lower levels of endotoxin-stimulated TNF- α production are associated with poorer outcomes in ICU patients (16,21,22).

The phenomenon of endotoxin/TLR tolerance is thought to play an important role in the susceptibility to reinfection in patients with severe sepsis (20).

Immunodepression in sepsis

The progress in understanding the molecular biology, genetics and epigenetics and the immune conditions of abdominal sepsis is beyond any doubt. Unfortunately, the postoperative incidence of septic complications after major visceral surgery remains high.

Surgical and trauma injury profoundly affects the innate and adaptive immune responses, and that marked suppression in cell-mediated immunity following an excessive inflammatory response appears to be responsible for the increased susceptibility to subsequent sepsis (21-26).

The immunosuppressive properties of the septic immune response are becoming increasingly relevant with continued

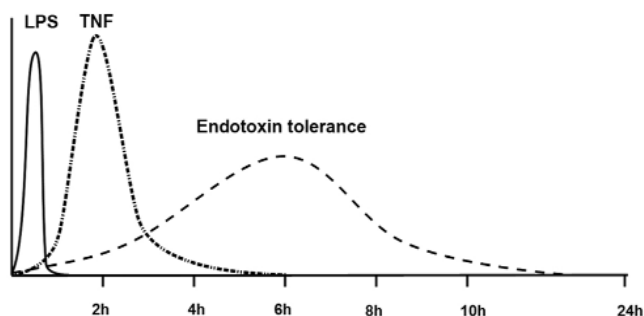


Figure 3. Time course of the plasma TNF levels after a single LPS stimulation in humans. "Endotoxin tolerance" is expressed as a "brake system" of the LPS signaling machinery [after (4,18,19)]

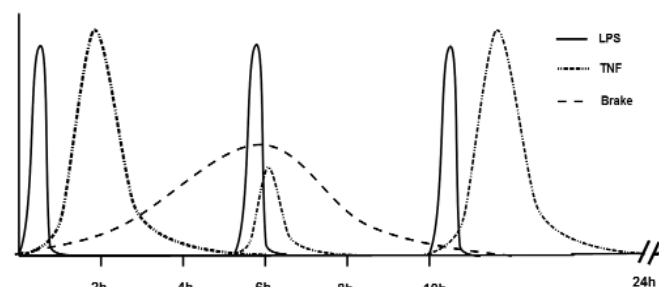


Figure 4. Time course of plasma TNF after repeated LPS stimulations. "Endotoxin tolerance" is expressed as a "brake system" of the LPS signaling machinery [after (4,18,19)]

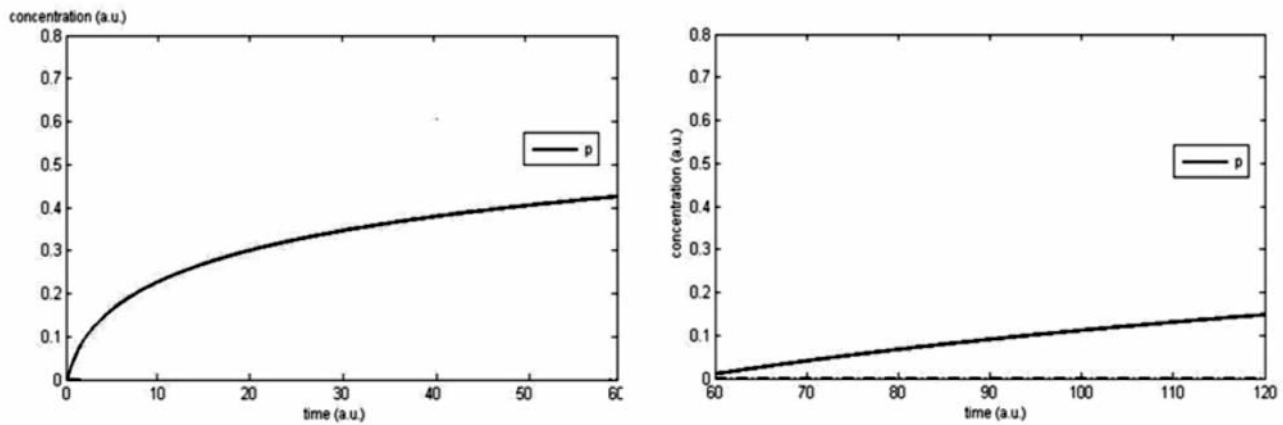
improvements in critical care. Many deaths due to sepsis do not occur acutely but rather occur after a prolonged hospital course, in patients with a marked innate immune hyporesponsiveness. In this condition the immunocompetent cells appear unable to release inflammatory mediators upon stimulation with bacterial antigens, mainly endotoxin (LPS). This may probably explain why therapies aimed at blocking pro-inflammatory mediators may be detrimental to septic hosts who are in a relatively immunosuppressed state (20).

Our broad understanding of the complex molecular pathways leading to activation of the immune response and the subsequent development of sepsis often creates the

impression that clear answers to both questions are available. However, the failure of most clinical trials of immunomodulators in sepsis indicates that we are far from understanding how the immune system functions when clinical sepsis appears (27). In previous works we established a mathematical model of LPS signaling based on endotoxin tolerance (28).

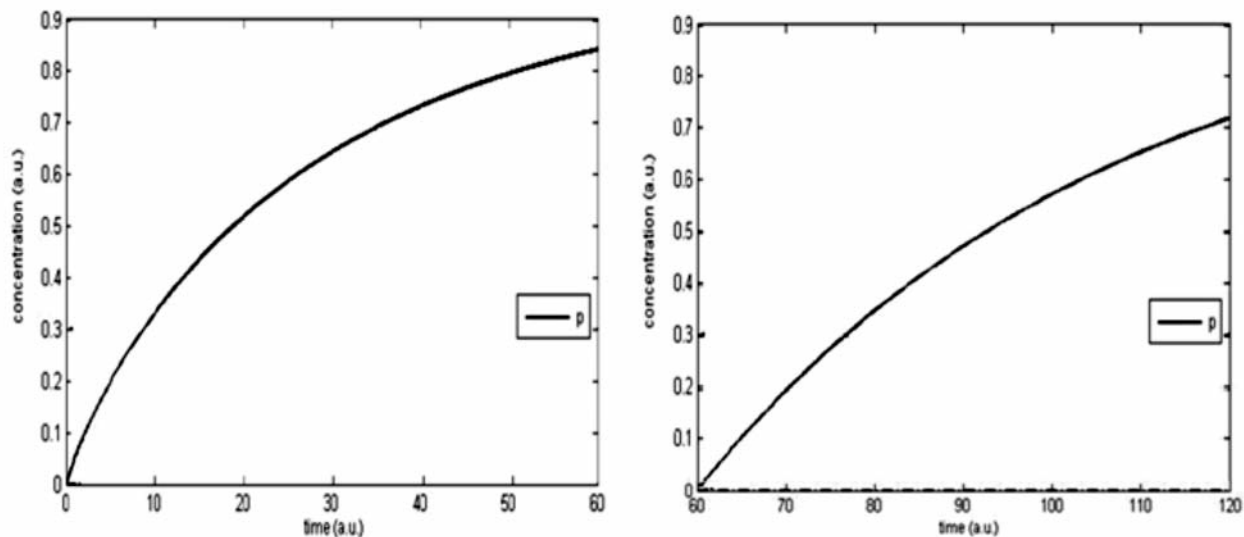
As a result of our simulations published in a recent paper (4) we conclude that inside cells the reaction kinetics is very well described by fractal-like kinetics. This type of enzyme kinetics may explain at least in part the preconditioning effect (29). (Fig. 5, Fig. 6)

Our hypothesis is that the fractal organization of the intra-



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).

Figure 5. Mathematical model of the TNF release after LPS stimulation; h is the so called "fractal parameter" [after (4)]



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).

Figure 6. Mathematical model of the TNF release after LPS stimulation; h is the so called "fractal parameter" [after (4)]

cellular environment imposes diffusion limits followed by fractal-like enzyme kinetics.

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

Acknowledgements

This paper was supported in part by the contracts 638/2009 UEFISCU and CEEEX 187/2006.

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