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Hypothesis

On the fractal nature of cytoplasm

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Abstract

On the basis of a quantification of the fractal dimension, D, in micrographs of cytoskeleton components or microtrabecular lattice, we propose that the cellular cytoplasm can be described as a *percolation cluster*, a sort of 'random fractal'. Our hypothesis deals with: (i) the existence of the *percolation threshold* – a remarkable property of percolation processes; and (ii) the reactivity increase – when enzymes, or targets, and substrates, or effectors, coexist in the same topological dimension.

Key words: Fractal dimension; Percolation cluster; Cytoskeleton; Heterogeneous fractal catalysis

1. Introduction

An emerging concept of the cytoplasm of the living cell is that of a structured, organized, macromolecular assembly. The organization inside a cell resembles that of a protein crystal with 40% water [1]. These conditions favour homo- and hetero-associations of enzymes or proteins, e.g. between microtubules and intermediate filaments, or even with subcellular organelles [2]. This complex cytoarchitecture has profound consequences for cell function. For instance, in *Escherichia coli* protein comprises 55% of the dry weight and about 36% of the dry weight of the cell is dedicated to protein synthesis [3,4].

The physical state, or more specifically, the rheological characteristics of the cytoplasmic aqueous domain, would influence a number of intracellular dynamic processes, including solute transport, diffusion-limited enzyme kinetics, and cell motility. It is widely accepted by biologists that cytoplasm cannot be considered as a simple Newtonian fluid [5,6]. A more realistic picture of the rheological nature of the cytoplasm approaches that of a *reversible, non-covalent gel network* [6,7] composed of complex supramolecular networks of actin filaments, microtubules, intermediate filaments and associated proteins.

A fractal view of the cytoplasm of living cells follows the 'structured' view but introduces new feasible behavioural possibilities with which we will be dealing in the present report.

2. The fractal structure of the cytoplasm as a percolation cluster, a non-euclidean cytostructure

Under the term *fractal* coined by Mandelbrot [8], different patterns are grouped which follow a geometry previously conceived as aberrant because they are undescribable from either a mathematical or geometric viewpoints. On the basis of a quantification of the fractal dimension, D, in micrographs of microtrabecular lattice, quick-frozen and deep-etched electron microscopy of axoplasm [2] or immunofluorescent images of the cytoskeleton through intermediate filaments and microtubules, we have recently proposed that the groundplan of living cells can be described as fractals of the type of *percolation clusters* [9].

One of the proposed definitions of fractals that contains its essential feature of *self-similarity* is: 'A fractal is a shape made of parts similar to the whole in some way' (Mandelbrot, quoted in [10]). In other words, fractal objects look the same whatever the scale, i.e. they remain invariant at several length scales. Fractals such as Cantor sets, Koch snowflakes, the Sierpinski gasket and Mandelbrot–Given curves, are constructed by applying a recursive rule, i.e. a *generator* on an *initiator* [8,10] (see Diagram A–D). For instance, in the Sierpinski gasket the *initiator* is a filled triangle and the *generator* eliminates a central triangle (Diagram C). A dimension,

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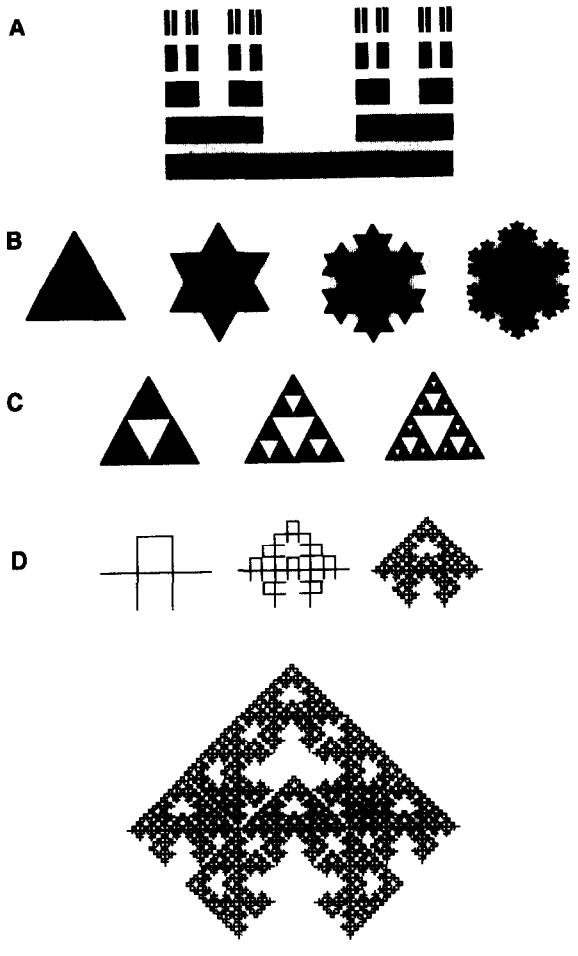

The first steps in the construction of (A) a Cantor bar; (B) a Koch snowflake; (C) a Sierpinski gasket and (D) a Mandelbrot–Given curve, whose fractal dimensions, D, are 0.67 (= ln 2/ln 3), 1.26 (= ln 4/ln 3), 1.58 (= ln 3/ln 2) and 1.89 (= ln 8/ln 3), respectively [10,21]. The Mandelbrot–Given curve has been used as a model of percolation clusters.

called the *fractal dimension*, D, characterizes fractal objects whose value does not correspond to those of the familiar dimensions 1,2 or 3 of euclidean geometry, i.e. lines, surfaces or volumes, respectively, but to an intermediate one (see legend Diagram). The fractal nature of the cytoplasm raises the possibility that the complex forms shown by cell's architecture, be explained from iteration of an invariant pattern that spans several length scales. Principles of fractal geometry have also been shown to apply to pulmonary airways and to blood vessels [11].

Elsewhere we have demonstrated that biological polymers such as (glyco)proteins and polysaccharides, can organize as fractals [9]. Perturbing hydrophobic or electrostatic interactions of polymers by physical or chemical methods, we have been able to induce diverse macroscopic morphologies. These morphologies arise from a lattice with different occupational probabilities and geometry which result in changes in the fractal dimension, D. The changes can be elicited likely by affecting bound water and polymer interactions [9]. Short-range interactions, e.g. van der Waals, electrostatic, hydrophobic, hydrogen bonds, are likely to participate in microscopic selection of different morphological features at the macroscopic level.

The finding that cytoskeleton components may be fractally organized as *percolation clusters* gives rise to the interesting possibility that the groundplan of living cells might behave according to the principles of fractal geometry.

3. Percolation clusters

A percolation cluster is the ensemble of pores or 'sites' in a lattice connected to a chosen center of injection of a fluid which will only invade another pore that is directly connected to that pore in a lattice through capillary channels or bonds [10]. The cluster that spans the lattice is called the spanning cluster or *percolation cluster*.

The most remarkable feature of percolation processes is the existence of a *percolation threshold*, p_c, below which the spreading process is confined to a finite region. The percolation probability, $P(p)$, is defined as the probability that a fluid injected at a site chosen at random will wet infinitely many pores. Below the *percolation threshold*, p_c, the cluster behaves as locally connected while above p_c the connection extend indefinitely [10] (see Appendix). Near the critical probability P_c, as the number of pores, p, is increased, *the percolation process undergoes a transition from a state of local connectedness to one where the connections extend indefinitely.*

A consequence of the considerations above, assuming that the cellular cytoplasm behaves according to the priciples of percolation processes, will be that *local* cytoplasmic behaviour when subjected to fluctuations or perturbations may extend and *globally* impose that behaviour to far remote regions in the cellular cytoplasm. This is a remarkable property since it would imply that above p_c, cytoplasmic activities may show coherent behavior, i.e. transitions from local (microscopic) to global (macroscopic). This coherence might be induced by fluctuations in local dynamics of biological processes, e.g. enzymatic fluxes, waves of second messengers or ions. When percolation processes are limited to finite regions, below the critical threshold, one may ask whether diffusion can be restricted. In percolation processes, the randomness of particle motion (e.g. metabolites, hormones) is associated *with medium properties*, e.g. sol or gel states. In contrast, in diffusion processes particles can spread indefinitely, the dynamics lying in the randomness of the Brownian motion [10]. Cytoplasm has the consistency of a viscoelastic gel the dynamics of which is ruled by reversible sol–gel transitions which induce different solvent properties of water [5]. Intracellular particles appear trapped within the gel or tethered to it with not evident

Brownian motion [6]. Consequently, diffusion in gel fractal networks is slowed down since there is a reduction in the number of diffusion paths available to a random walker, or obstruction by exclusion volume effects [6]. Available experimental evidence with fluorescent probes suggests that cytoplasm is intrinsically compartmentalized by spatial and temporal variation of its submicroscopic structure [6].

According to the view of the cytoplasm as a percolation cluster, one may easily imagine the following picture: metabolites, hormones, second messengers, ions, would percolate through the macromolecular network with the striking possibility that the processes (or the targets) in which they are locally involved, become connected and synchronous to similar processes occurring far remote in the cytoplasm or targets distributed further away from each other.

4. Catalysis in heterogeneous fractal media

The enhanced performance of catalysts in heterogeneous fractal media may be understood through the simultaneous operation of two mechanisms. The first one is based on the shredded topology exhibited by percolation clusters. For reactions in heterogeneous media, the larger the interface the faster the reactions, i.e. the rate per unit surface is constant. The latter is known as Wenzel's law (quoted in [14]). For shredded topologies at equal length of rims with respect to connected topological arrangements, the reactions proceed faster in the former. This violates Wenzel's law [14]. It has been proposed that the increase in reactivity per unit surface may be due to segregation of reactants since a higher segregation is expected on the disjointed (shredded) catalyst arrangement [14].

The second mechanism deals with the transitions from 2D to 3D dimensions or vice versa occurring in fractal objects, i.e. an area may fractally evolve into a volume or a volume by fractal folding may attain the properties of an area [15]. The latter is crucial since it has been known for a long time that the probability of a random walker (e.g. a substrate) to find its target (e.g. enzyme) is higher in 2D than in 3D dimensions (Polya, 1921: quoted in [16]). Substrate or effector, and enzyme or target, may find each other in the same 2D dimensional space which increases the encounter probability between them.

The catalytic properties of enzymes at the cellular level are expected to be influenced by the fractal nature of the cytoplasm. In fact, a percolation cluster is a highly shredded, disjointed, object where the reactivity per unit surface will increase respect to a connected geometry. In single- or two-reactant bimolecular reactions, it has been shown that the increase in reactivity per unit area may be explained by reactants segregation [14]. The increase in probability for a substrate to find its target is equivalent to the increase in reactivity when local reduction of dimensions occurs in fractally folded spaces. In other words, increase in reactivity will occur when enzymes, or targets, and substrates, or effectors, coexist in the same topological dimension.

5. Discussion

One of the main suggestions of the present report is that the ground substance of living cells might behave according to the properties of percolation clusters. One main consequence of the fractal nature of the groundplan of living cells would be its effects on catalysis. Existing experimental evidence [17] points to the fact that polymers able to fractally fold in space have profound effects on the dynamics of systemic cellular properties such as metabolic fluxes.

Another consequence of the cytoplasm behaving as a percolation cluster would be that the effect of exogenous substances (e.g. inhibitors) affecting cellular widespread cytoplasmic elements such as the cytoskeleton, should exhibit *threshold behavior*. More explicitly, we are suggesting that the *percolation threshold* may be related to the well known *lethal dose* operationally defined as the concentration at which inhibitors or pharmacos exert 50% of their maximum effect. Percolation fractal lattices provide a conceptual framework to interpret existing experimental evidence related to cells treatment with several microtubule disrupting agents [12,13].

How could an extracellular stimulus (e.g. light, hormones, pH, oxygen) induce a coherent answer at the cellular level that induces the cell to different developmental paths, to stop division or to redirect metabolic fluxes? We have argued elsewhere [18] that at the cellular level, the passage from microscopic to macroscopic coherence would be given by instability of the dynamics of processes like polymerization–depolymerization of cytoskeleton components. One main item of evidence that led us to suggest the autonomous dynamics of cytoskeleton as affecting higher levels of organization, was that its spatio-temporal 'window' (micrometers and a few minutes) of occurrence happened within the domain likely to give rise to macroscopic coherence at the cellular level, i.e. microtubules extend several micrometers in the lapse of minutes [19,20]. Instabilities in the autonomous dynamics of biological processes in a far from thermodynamic equilibrium domain, give rise to self-organized behavior. This behavior could in turn synchronize collective or systemic properties in cells such as metabolic fluxes [17].

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APPENDIX

Percolation clusters

A remarkable feature of percolation processes is the existence of a percolation threshold, p_c, below which the spreading process of a fluid is confined to a finite region [10]. The percolation probability, $P(p)$, is defined as the probability that a fluid injected at a site chosen at random will wet infinitely many pores [10]. For low concentrations, p, of wet pores we find that $P(p)$ is negligible. As p increases, the probability of belonging to the largest cluster increases drastically near $P_c = 0.593$ (for a quadratic lattice) and then $P(p)$ increases almost linearly to 1 as $p \rightarrow 1$. The critical probability, P_c, is defined as the largest value of p for which $P(p) = 0$. Thus, by definition we have $P(p) = 0$ for $p < P_c$.

Near the critical probability, P_c, as p is increased, the probability of belonging to the largest cluster increases drastically. The *percolation process undergoes a transition from a state of local connectedness to one where the connections extend indefinitely* [10].

The percolation probability vanishes as a power-law near p_c:

$$P(p) \sim (p - p_c)^{\beta}, \text{ for } p > p_c, \text{ and } p \rightarrow p_c$$

This is analogous to what happens at magnetic phase transitions, where the local order or magnetic moment increases in range as the temperature is lowered to the transition temperature, Tc, of the material [10].