The Fractal Structure of Glycogen: A Clever Solution to Optimize Cell Metabolism

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ABSTRACT   Fractal objects are complex structures built with a simple procedure involving very little information. This has an obvious interest for living beings, because they are splendid examples of optimization to achieve the most efficient structure for a number of goals by means of the most economic way. The lung alveolar structure, the capillary network, and the structure of several parts of higher plant organization, such as ears, spikes, umbels, etc., are supposed to be fractals, and, in fact, mathematical functions based on fractal geometry algorithms can be developed to simulate them. However, the statement that a given biological structure is fractal should imply that the iterative process of its construction has a real biological meaning, i.e., that its construction in nature is achieved by means of a single genetic, enzymatic, or biophysical mechanism successively repeated; thus, such an iterative process should not be just an abstract mathematical tool to reproduce that object. This property has not been proven at present for any biological structure, because the mechanisms that build the objects mentioned above are unknown in detail. In this work, we present results that show that the glycogen molecule could be the first known real biological fractal structure.

INTRODUCTION

Glycogen is a highly branched structure that plays the metabolic role of a quick fuel supplier. Thus, its structure must guarantee the availability of a large amount of glucose in a short time to account for quick movements. Details of its structure and synthesis pathways can be seen in Fig. 1, drawn according to data from Gunja-Smith et al. (1970), Goldsmith et al., (1982), and Meléndez-Hevia et al. (1993). Release of stored glucose from glycogen is catalyzed by phosphorylase, which yields units of glucose 1-P by attacking the glycogen external chains with inorganic phosphate from the nonreducing ends. This enzyme is activated through a cascade mechanism, which accounts for a rapid and large fuel availability (see a review in Johnson, 1991). Cárdenas and Cornish-Bowden (1989) have shown that such a regulation mechanism produces a kinetic behavior of a highly cooperative effect with a Hill coefficient that can be up to 800, which is practically a switch. The full molecule of glycogen, also called macroglycogen, has 12 tiers, but only the four most external ones are usually involved in the regular turnover of the synthesis–degradation cycle. The skeleton formed by the eight inner tiers (called proglycogen) only contains ~5% of the stored fuel (Lomako et al., 1991).

Previous studies by our group using mathematical modeling (Meléndez-Hevia et al., 1993; Meléndez et al., 1997, 1998) showed that several structural aspects of the glycogen molecule are optimized to achieve an efficient fuel storage molecule. The features of glycogen structure were described by four variables: the number of external chains (C_A), the total number of glucose residues stored in the molecule (G_T), the number of residues directly available to phosphorylase (G_{PT}), and the volume of the molecule (V_S). They depend on three parameters: the branching degree (r), the number of tiers (t), and the number of glucose residues in each chain (g_c). The mathematical model that describes the molecular structure is described by the set of equations,

$$C_A = r^{t-1}, \tag{1}$$

$$G_T = g_c \cdot \frac{1 - r^t}{1 - r}, \tag{2}$$

$$G_{PT} = C_A \cdot (g_c - 4), \tag{3}$$

$$V_S = \frac{4}{3} \pi \cdot t^3 \cdot (0.12 \, g_c + 0.35)^3. \tag{4}$$

The optimized structure of glycogen was found maximizing the function,

$$f_{opt} = \frac{G_T \cdot C_A \cdot G_{PT}}{V_S}, \tag{5}$$

which means to store the maximum amount of glucose directly available to phosphorylase in the most possible dense molecule. In previous works, we have demonstrated that the values of the three parameters of cellular glycogen are those that optimize this function. This was shown for each parameter, namely the chain length $g_c = 13$ (Meléndez-Hevia et al., 1993), the branching degree, with $r = 2$ (Meléndez et al., 1997), and the maximum number of tiers with $t = 12$ (Meléndez et al., 1998). Now we shall show that the glycogen molecule has a fractal structure that accounts
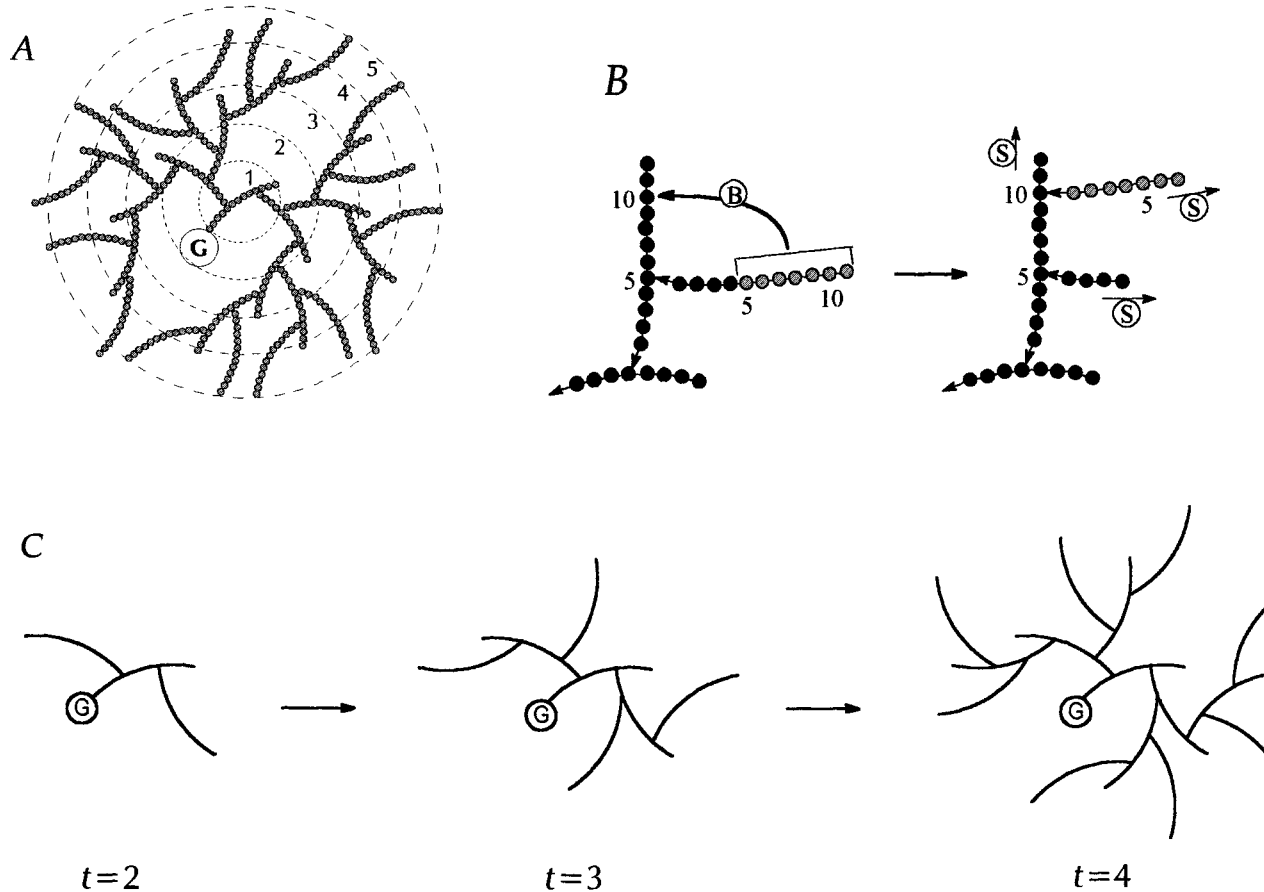
FIGURE 1 Glycogen: structure and mechanism of synthesis. (*A*) Glycogen structure according to Whelan's model (Gunja-Smith et al., 1970; and more data in Goldsmith et al., 1982; Meléndez-Hevia et al., 1993). The molecule has a spherical shape with 12 concentric tiers ($t = 12$) in the full molecule (only 5 are drawn in the picture). It is formed by chains of glucose polymerized by $(1 \rightarrow 4)$ glycosidic bonds, with an average length of $g_c = 13$ glucose residues per chain, with two branching points ($r = 2$) by means of $(1 \rightarrow 6)$ glycosidic bonds generating new chains. In the degradation of the molecule, phosphorylase releases glucose residues from the nonreducing ends of the external (nonbranched) chains, supplying the fuel that can be used immediately. Many glycogens from different sources showed to have the same values of the parameters and to have a homogeneous structure $r = 2$, $t_{max} = 12$; $g_c = 13$. (*B*) Iterative mechanism of glycogen synthesis: Glycogen synthase (S) promotes the growth of the chains adding glucose units by $1 \rightarrow 4$ bonds. When a chain has at least 11 residues, the branching enzyme (B) transfers a stretch of 7 glucose residues to another chain making $1 \rightarrow 6$ bonds between glucoses, and producing the growth of new branches. The new branch point must be at least at the fifth residue from a preexisting one. Then, glycogen synthase promotes the growth of every chain again, and the process is repeated (see Manners, 1968; Alonso et al., 1995). (*C*) General view of molecular growth by means of synthesis of new tiers. The value of t is increased in each iteration.

for the optimization of other important properties concerning its metabolic role.

APPROACH AND RESULTS

Fractal properties of glycogen

From a mathematical point of view, a fractal object is a structure made by an infinite iteration of a building process. So extensive a succession is obviously not possible when the object has a physical entity. Thus, to consider whether a certain physical structure is fractal must be done by studying whether it fulfills the fractal properties inside a discreet range.

The branched structure of glycogen (Fig. 1) seems to suggest that the molecule may have fractal properties, because it is the result of a typical iterative process characteristic of a fractal structure. Let us check whether it fulfills the typical features of fractal structures.

Self reference

Self reference is the application of a simple recurrent algorithm that allows us to achieve a complex structure. This is analytically proven for a number of mathematical fractal objects, such as Cantor's set or Koch's curve. However, until present, the existence of such fractal structures in nature is just a mathematical concept, because there is no proven relation with a similar biological procedure.

Since the first description of fractal structures mathematically, many authors have been trying to demonstrate the fractal condition of several biological objects. A number of structures present in living beings, such as the lung alveolar

TABLE 1 Set of equations to calculate structural features of the glycogen molecule

Necessary number of hexagons to cover the full surface
$$N(k) = N(t) = C_A = 2^{t-1}$$

Radius of the molecule (nm)
$$R_S = (0.12 \cdot g_c + 0.35) \cdot t$$

Surface of the molecule (nm²)
$$S_{glycogen} = 4 \cdot \pi \cdot R_S^2$$

Surface of each domain (nm²)
$$S_{hexagon} = \frac{S_{glycogen}}{C_A}$$

Radius of the hexagon
$$R = \sqrt{\frac{2 \cdot S_{hexagon}}{3 \cdot \sqrt{3}}}$$

Effective distance between two domains
$$\epsilon = R \cdot \sqrt{3}$$

Details about number of chains (C_A) and radius of the molecule (R_S) can be seen in (Meléndez-Hevia et al., 1993).

structure, the capillary network, and others mentioned above, are supposed to be fractals because they have a fractal shape (see, e.g., Mandelbrot, 1983; Goldberger et al., 1990). Such a statement is, however, intuitive, because, although mathematical functions based on fractal geometry algorithms can be developed to simulate such structures (see Guzmán et al., 1993; Barnsley and Demko, 1985; Weibel, 1991), an iterative process that accounts for their building in the living beings is not known. The statement that a given biological structure is a fractal should imply that the iterative process of its building has a real biological meaning, i.e., that its construction in nature has been achieved by means of a single genetic, enzymatic, or biophysical mechanism successively repeated. Although the shapes of such structures suggest that they could have been built in nature by means of such a fractal single mechanism successively applied, an analytical study of their growth rules is not yet available (see Guzmán et al., 1993).

In the case presented here, we have an detailed knowledge of how a tree-like fractal structure is built in nature. The apparently complicated structure of glycogen is built by means of a simple mechanism involving only two enzymes: glycogen synthase and the branching enzyme (see Fig. 1), and it is really possible to make glycogen in vitro, controlling every tool that intervenes (Smith, 1968; Krisman et al., 1985).

Self-similarity

Self-similarity states that the object has the same appearance independently of the magnifying degree with which one can see it; that is, a fractal structure must have detail at any scale from which it is being observed. This property is fulfilled regarding the domains for phosphorylase attack on the surface of the molecule, as Fig. 2 shows. Each iteration affects all external chains, creating a new external tier that duplicates the number of external chains, and so, the num-
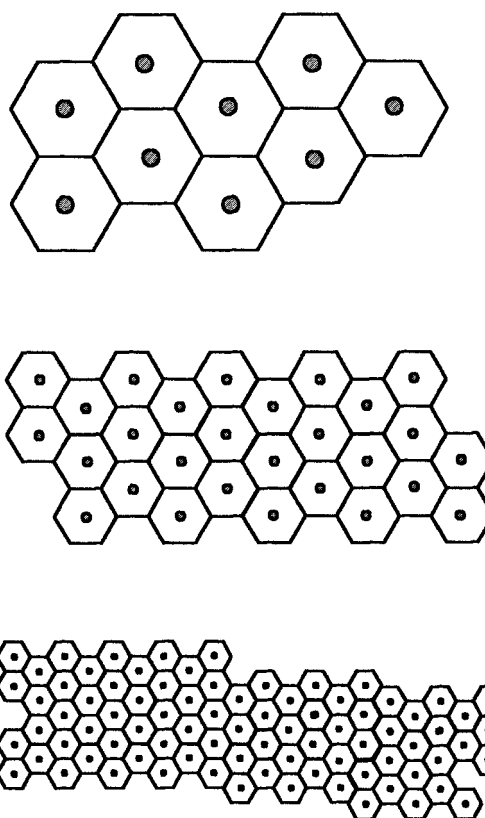

FIGURE 2 The set of the attack domains for phosphorylase. In each iteration, the number of domains increases exponentially in a limited surface. In this picture, the domains are represented by hexagons.

ber of points of attack for phosphorylase. If all chains of the last tier from a full molecule of glycogen are removed, a series of more separate chain ends covering the new spherical surface is obtained, so the phosphorylase domains are larger. Then, by removing that tier, another spherical surface with the same picture appearance is found, and the process can be repeated as many times as the number of tiers the glycogen molecule has, the same picture being always obtained. Looking at a given part of the surface, one can see that only the size of the domains changes with each iteration.

Fractal dimension

The fractal dimension informs us about the compacticity of a fractal structure. We have calculated it by means of the box counting method described by Falconer (1990) as follows: Let us assume that the glycogen molecule is a sphere on which the ends of the glycosidic chains are homogeneously distributed. We can represent the domains for phosphorylase attack as regular hexagons (Fig. 2). Since the molecule has a regular structure, i.e., the chain ends are regularly distributed, the attack domains are all equal, and they cover the whole surface (Fig. 3). We have thus a surface covered by hexagonal boxes. The effective distance between two chain ends is twice the apothem of the hexa-
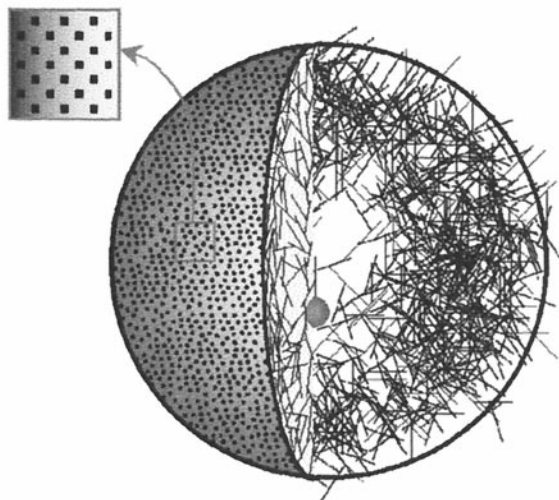
FIGURE 3   General view of the glycogen molecule. Glycogenin, the small protein, primer for the molecule synthesis, is in the core. The cut allows us to see the interior. The nonreducing ends, terminals of the last tier, form a surface where the enzymes can work.

gon, and this length will depend on the tier we consider, because it is smaller with each iteration k.

Let $N(k)$ be the number of boxes that recover the whole structure, and ϵ the diameter of the boxes. So, according to the expression of the fractal dimension,

$$D_f = \lim_{k \to \infty} \frac{\log N(k)}{-\log \epsilon} \qquad (6)$$

because the number of domains is the number of external chains of glycogen $N(k) = C_A$, ϵ is the effective distance between two ends (twice the apothem of the hexagon). We need now to express $N(k)$ and ϵ as a function of the number of tiers, as t (tiers) $= k$ (iterations), to calculate the limit in Eq. 6.

According to the equations in Table 1, ϵ can be expressed as a function of the number of tiers t as

$$\epsilon = q \cdot \frac{t}{2^{(t-1)/2}} \qquad (7)$$

where q is a constant that includes all other parameters. Thus, the fractal dimension is calculated by

$$D_f = \lim_{t \to \infty} \frac{N(t)}{-\log \epsilon} = \lim_{t \to \infty} \frac{\log 2^{t-1}}{-\log\left(k \cdot \dfrac{t}{2^{(t-1)/2}}\right)} = 2. \qquad (8)$$

It is thus $D_f = 2$. This means that, although the number of glycosidic chain ends (points for phosphorylase attack) is an object of dimension zero, in the limit of the infinite iteration, it increases until covering the surface of the spherical molecule (see Fig. 3). At that point, this set would become a surface. Since the fractal dimension of this set could be between 0 and 2 (a set of 0-dimensional objects distributed in a 2-dimensional space), this result shows that glycogen

mechanism of synthesis generates a structure with the maximum possible compacticity, which is an obvious target of optimization. It is clear that the number of iterations cannot be infinite. The full molecule (macroglycogen) has 12 tiers, and only the last four are usually involved in the regular turnover of synthesis-degradation. However, the set of non-reducing ends in the four external tiers behaves as a surface for the enzymes, making it possible that phosphorylase slides on it to be able to find quickly the next point of attack.

DISCUSSION

Why and how is glycogen fractal? The two basic properties of fractal structures, self-similarity and self-reference, are also, respectively, the answers to the two general questions about biological structures: why and how. The first question is which metabolic advantages are derived from the fact that the glycogen structure is fractal?; and the second one is which is the cell procedure able to achieve such a structure? As to the first question, there is a number of clear advantages to such a structure that contribute to optimize its role in cellular metabolism:

1. Simplicity in the synthesis and degradation mechanisms.—A fractal structure such as the glycogen molecule involves a very easy pathway of synthesis and degradation. This is a key metabolic feature because it allows rapid building and degradation of the molecule, allowing a quick release of the stored fuel, as well as a fast recovery, the glycogen molecule being ready promptly for the next use. A simple short pathway favors a high metabolic flux, as has been demonstrated by Meléndez-Hevia et al. (1994).

2. Simplicity in the regulation mechanism.—An interesting consequence derived from the above one is that, because the mechanism to build the glycogen molecule is very simple, its regulation can also be easy. It gives us another example of simplicity as an evolutionary target of metabolic optimization (Meléndez-Hevia et al., 1985, 1988, 1994).

3. The optimization process of its structure is easier.—Since the glycogen molecule is built by means of a simple iterative procedure, its optimization is easy, because there are few parameters and variables to bear in mind. The constraints of the optimization have been previously described by means of mathematical modeling and checked empirically (Meléndez-Hevia et al., 1993; Meléndez et al., 1997, 1998). Such an optimization process involves both the structure and the pathways of its metabolism.

4. The fractal properties are extended to other variables.—Every variable that is linear with respect to the number of chain ends is also fractal. This applies to the glucose directly available for phosphorylase (G_{PT}) and the time the energy metabolism can work supported by it. This property accounts for a number of features of the glycogen molecule that are independent from its size, i.e., that make the molecule functional at any moment of its synthesis or degradation. For example, when the most external tier is spent, one finds a similar structure, and thus, the glucose

directly accessible to phosphorylase is always 33% of the total amount of the stored glucose independent of the size of the molecule.

The fractal structure also has important consequences on the stability of the molecule. The spherical shape of the glycogen molecule—a consequence of its fractal structure—guarantees a homogeneous symmetrical shape. This leads, on the one hand, to the maximum potential energy in the surface, as the amount of convertible glucose units. On the other hand, the surface homogeneity maximizes the number of hydrogen bonds among glucose residues, increasing the stability of the molecule despite its high energy content. This determines an interesting problem concerning molecular energy that we shall explore in a future work.

In general, fractal structures in nature could be classified in two different classes: useful and curious fractals. The first class includes objects with a clear relationship between their fractal structure and their function, such as the blood capillary network, the alveolar organization of lung, or the umbel inflorescence, provided that they are fractals. In contrast, there are also objects whose fractal structure is only a geometrical property, and its performance does not depend on it. Examples included in this group are, for example, the coast of Britain, the tree bark, or the cell membrane. All of them have been described as fractal structures (see, e.g., Mandelbrot, 1983; Goldberger, 1990), but such a feature is not the consequence of a highly organized structure, but a property of the material per se. For example, membranes are fractal because of the physical properties of the materials from which they are made—it is really not possible to build a nonfractal membrane as it is not possible to find a nonfractal coast. It is possible to build this kind of fractal found in nature following an iterative mathematical algorithm (see Mandelbrot, 1983; Goldberger et al., 1990; Guzmán et al., 1993; Weibel, 1991; Barnsley and Demko, 1985), but this does not have any biological meaning, because the pathway of membrane synthesis, for example, yields directly such a structure without involving an iterative process. The fractal quality of such structures is thus only a curious geometrical property. Glycogen is a useful fractal; it can be considered a true fractal because its geometrical properties have a biological meaning, it is built by the enzymes through an iterative process, and its optimized structure strongly depends on such fractal properties.

We have seen that there is no doubt about the multiple advantages that fractal structure provides to glycogen, the question of why glycogen is a fractal is thus answered. Concerning how the cell is able to build such a structure, the alternate intervention of synthase and branching enzymes (Fig. 1) explains the iterative operation (algorithm) that works at each level, building each tier. However, such a mechanism itself cannot be enough to guarantee the final homogeneous molecule of glycogen. It is easy to understand that, if there are no restrictions on the activity of the two enzymes, both of them would preferentially work on the external chains promoting mainly only their growth, and leaving many internal chains unfinished, which is not the structure observed experimentally (see, Lomako et al., 1991; Alonso et al., 1995; Meléndez et al., 1997). The mechanism of glycogen synthesis should thus work under certain constraints to guarantee the homogeneity of the end product. The alternate action of the enzymes, together with such constraints, is the key to answer the means by which procedure the cell achieves glycogen fractal structure.

We have analyzed which these constraints should be, and our proposal is the following. 1) The branching enzyme should be in a high excess over synthase; this would mean that a new branch was made every time that it was physically possible, according to the requirements of the enzyme mechanism (Fig. 1). 2) The process of glycogen growth should be favored in the more internal zones; i.e., both synthase and branching enzymes should preferentially work in an inner zone, provided that the density of that zone allows it, to avoid the excessive growth of the external chains when those in the inner tiers are not yet finished, which would result in an inhomogeneous molecule. 3) Phosphorylase working in the regular direction, i.e., degrading glycogen chains, should also function during the biosynthesis process, to correct any mistake of the above conditions, removing those chains that are too external and thus allowing synthase and branching to repair the inappropriate structure, completing the unfinished tiers. These three conditions are theoretical predictions of the model, and they also have empirical support. In effect, it has been previously shown that activity of the branching enzyme is ~200 times over synthase's (see Meléndez et al., 1997). In contrast, Meléndez et al., (1998) have shown by mathematical modeling that, unless some restriction is imposed in the mechanism of the synthesizing enzymes, they tend to promote the chain growth and branch building on the more external tiers, leaving wide internal zones unfinished. This strongly suggests that these enzymes should have more affinity for working in internal zones of the molecule to produce the homogeneous molecule well demonstrated by experimental analysis.

Until now, phosphorylase has been only recognized as the key tool in glycogen degradation. Here, we are proposing a new role for it in the mechanism of glycogen biosynthesis, with only the small remaining activity at the resting state of the tissue, when the cascade activation has not been triggered. Such a remaining activity of phosphorylase could be very useful to give more opportunities to both synthase and branching enzyme to fill the inner tiers, and so to achieve a better complete and homogeneous molecule. As a matter of fact, there are many experimental data showing that both enzymes, synthase and phosphorylase, work together during the process of glycogen synthesis (see David et al., 1990; Meléndez-Hevia et al., 1997). This fact was initially interpreted as a futile cycle in glycogen synthesis. Now, according to this new view, such a cycle would make sense as a regulation mechanism to achieve a highly homogeneous molecule.

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