

## Physical Constraints in the Synthesis of Glycogen That Influence Its Structural Homogeneity: A Two-Dimensional Approach

Ruth Meléndez,\* Enrique Meléndez-Hevia,# Francesc Mas,§ Jordi Mach,§ and Marta Cascante\*

\*Departament de Bioquímica i Biologia Molecular and §Departament de Química Física, Facultat de Química, Universitat de Barcelona, 08028 Barcelona, and #Departamento de Bioquímica, Facultad de Biología, Universidad de La Laguna, 38206 Tenerife, Canary Islands, Spain

**ABSTRACT** Several aspects of glycogen optimization as an efficient fuel storage molecule have been studied in previous works: the chain length and the branching degree. These results demonstrated that the values of these variables in the cellular molecule are those that optimize the structure-function relationship. In the present work we show that structural homogeneity of the glycogen molecule is also an optimized variable that plays an important role in its metabolic function. This problem was studied by means of a two-dimensional approach, which allowed us to simplify the very complicated structure of glycogen. Our results demonstrate that there is a molecular size limit that guarantees the structural homogeneity, beyond which the structure of the molecule degenerates, as many chains do not grow. This strongly suggests that such a size limit is precisely what the molecule possesses in the cell.

### INTRODUCTION

Glycogen is probably the only case in cellular metabolism where a relationship between structure and metabolic function has been mathematically proven (Meléndez-Hevia et al., 1993). A schematic picture of the glycogen molecule is shown in Fig. 1, drawn according to Whelan's model (Gunja-Smith et al., 1970) and additional data (Goldsmith et al., 1982; Meléndez-Hevia et al., 1993). It is formed by chains of glucose polymerized by (1 → 4) glycosidic bonds, each one having an average length of 13 glucose residues with two branching points by means of (1 → 6) glycosidic bonds generating new chains; only the most external ones (A-chains) are not branched. The molecule has a spherical shape, organized in concentric tiers, with the full molecule having 12 tiers.

Several structural aspects of glycogen have been shown to be optimizable to achieve an efficient fuel storage molecule. This has been studied previously (Meléndez-Hevia et al., 1993) by means of mathematical modeling. That study showed that the structural optimizable properties of the glycogen molecule depend on three parameters, namely, the branching degree ( $r$ ), the number of tiers ( $t$ ), and the number of glucose residues in each chain ( $g_c$ ). Glycogen structure was described by four variables: the number of external chains also called A-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 ( $V_S$ ). The mathematical model of

the molecular structure was described by the following set of equations:

$$C_A = r^{t-1} \quad (1)$$

$$G_T = g_c \frac{1 - r^t}{1 - r} \quad (2)$$

$$G_{PT} = C_A (g_c - 4) \quad (3)$$

$$V_S = \frac{4}{3} \pi r^3 (0.12 g_c + 0.35)^3 \quad (4)$$

The values of the parameters that optimize the structure of glycogen were found by maximizing the optimization function:

$$f_{opt} = \frac{C_A G_T G_{PT}}{V_S}, \quad (5)$$

which means to store the maximal possible amount of glucose directly available to phosphorylase in the most highly compact molecule (the highest density).

The analysis of Eq. 5, plotted in Fig. 2, demonstrated that the values of the parameters that cellular glycogen has are those that maximize its optimization function: branching degree  $r = 2$  and chain length  $g_c = 13$  (Meléndez et al., 1997; Meléndez-Hevia et al., 1993). This is supported by data of glycogen structure obtained by us and previously reported by other groups, which show that these values of the parameters have been achieved by a broad number of species and groups, including vertebrates, invertebrates, yeast, bacteria, and protozoa (see a selection of data reviewed in Meléndez et al., 1997).

The size of the glycogen molecule was shown also to be the same in a broad number of sources, with a molecular weight of  $10^7$ , which includes 55,000 glucose residues in 12 tiers (see Goldsmith et al., 1982; Meléndez-Hevia et al., 1993). The aim of this work was to investigate why glyco-

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Address reprint requests to Prof. Enrique Meléndez-Hevia, Universidad de La Laguna, Departamento de Bioquímica, Facultad de Biología, 38206 Tenerife, Canary Islands, Spain. Tel.: 34-922-630126; Fax: 34-922-630126; E-mail: emelhev@ull.es.

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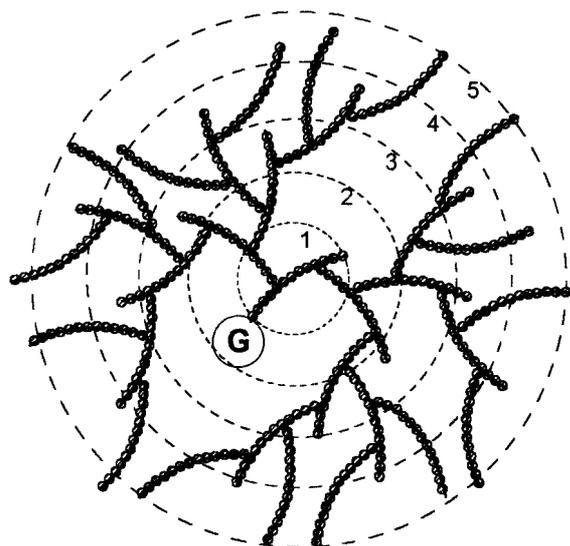


FIGURE 1 Glycogen structure. Scheme showing a general view of the cellular glycogen molecule, drawn according to Whelan's model (Gunja-Smith et al., 1970) and additional data (Goldsmith et al., 1972; Meléndez-Hevia et al., 1993). The values of the parameters are as follows: branching degree  $r = 2$ , chain length  $g_c = 13$ , and number of tiers  $t = 12$ ; only 5 tiers (marked with dashed circles) have been drawn to simplify the picture. G, glycogenin, a small peptide which is the primer for glycogen synthesis.

gen has such a size. We have carried out this analysis by means of a two-dimensional (2D) approach, developing a computer program that constructs plane glycogen molecules. This allowed us to investigate the synthesis of glycogen molecules beyond the regular limits where the building of the cellular glycogen is stopped. We have found that those glycogens that would surpass the regular size that they have in the living cell would lose the structural homogeneity and would become poorly optimized molecules. We thus conclude that the size of glycogen is also an optimizable variable, and our results strongly suggest that the size that the molecule has in the cell is the optimum to have the maximal amount of glucose stored without a significant loss of homogeneity.

### Size and homogeneity of the glycogen molecule

The building of the glycogen molecule is carried out by means of the coordinated action of two enzymes: glycogen synthase (EC 2.4.1.11) and the branching enzyme (EC 2.4.1.18). The mechanism of synthesis is shown in Fig. 3. Glycogen synthase builds the polymer by adding glucose residues from the activated monomer UDP-glucose, and the branching enzyme cuts a stretch from a long chain and attaches it to another, forming a new branch (see reviews in Calder, 1991; Alonso et al., 1995).

It can be considered that two parts form the full glycogen molecule with 12 tiers, also called macroglycogen: the inner part, which includes the first eight tiers, called proglycogen, and the external part, which includes the four external tiers. Only the four external tiers are usually involved in the

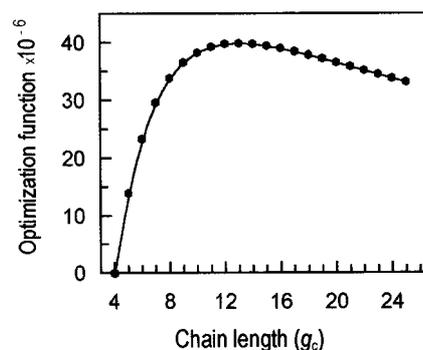


FIGURE 2 Optimization function of the cellular (3D) glycogen. Plot of the optimization function of cellular glycogen structure (Eq. 5), according to the value of the chain length ( $g_c$ ). The function is maximum for  $g_c = 13$ , which is precisely the value of this parameter in cellular glycogen from many different sources.

regular metabolic turnover of glycogen synthesis and degradation as energy fuel. Proglycogen, which contains only  $\sim 6\%$  of the glucose stored in the full glycogen, plays mainly a molecule skeleton role, providing the basic structure for glycogen to continue its growth, and only in extreme cases can its stored glucose be used as energy fuel. The enzymes that work in each stage of the synthesis of the full molecule are different: proglycogen synthase and macroglycogen synthase, which have been described as two different isoenzymes (Alonso et al., 1995).

The cascade mechanisms that regulate the activity of enzymes involved in glycogen synthesis and degradation are well known (see a review in Hubbard and Cohen, 1993). However, the mechanism controlling cessation of biosynthesis when a full size is reached is still an unanswered question. Madsen and Cori (1958) realized that, as the branching degree is constant in all tiers, the number of chains increases as the molecule grows, and so the density of the molecule is higher in the more external tiers. This led them to suggest that the glycogen structure could be self-limiting in size. This hypothesis is at present the only statement about this subject (see Goldsmith et al., 1982; Meléndez-Hevia et al., 1993; Meléndez et al., 1997).

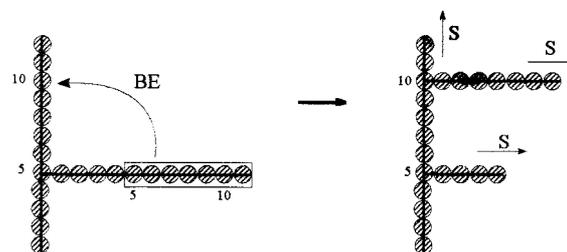


FIGURE 3 Mechanism of building the glycogen molecule. Glycogen is synthesized by means of the coordinated action of two enzymes: synthase (S) promotes the chain growth by adding glucose residues on a previously existing chain. The branching enzyme (BE) produces new branches transporting a stretch of a chain (seven residues) to another point of the molecule. Four glucose residues must at least remain between two branching points.

But why does the glycogen molecule have precisely 12 tiers? Let us assume that it is controlled by Madsen and Cori's hypothesis; then the size of the synthesizing enzymes is what determines the size of the molecule, as the synthesis is stopped when the density is so high that there is not enough room for these enzymes. Could the enzymes be smaller, and then could the glycogen have more tiers? Is then the size of the glycogen molecule optimized? In other words, is the size of the molecule constrained by the smaller possible size of the enzymes or is there an optimal limit in the number of tiers ( $t_{\max}$ ), independent of the enzyme size, and has the enzyme size been adapted to stop the growth at that point? This question is not obvious because the optimization function (Eq. 5) is independent of  $t$  (see Meléndez-Hevia et al., 1993).

A necessary condition for the good functioning of Madsen and Cori's mechanism is that the glycogen molecule has a high structural homogeneity, as otherwise it would be effective only on certain parts of the molecule, leading to an irregular growth. This mechanism requires that the external chains are homogeneously distributed in the last tier, and this can be well achieved because there are many binding sites with a high mobility, so the chains tend to move away from each other as much as possible. This property is enhanced in a glycogen with many chains.

As the mechanism of synthesis is based on cuttings and rebindings that operate on statistically distributed points of the external tier of glycogen (see Fig. 3), the building of a homogeneous glycogen is obviously easier when more space is available for the synthesis process. If the enzymes had to work on an inhomogeneous ground, with some zones crowded and others emptier, the growing of the molecule would be preferentially developed on the wide zones, producing an irregular structure. This would be a poorly optimized glycogen because its metabolic functioning depends on the maximal available glucose in each tier, which is only achieved if every tier is full, i.e., if the branching degree  $r = 2$  is constant on every chain and in all tiers. If this property were not fulfilled, and some chains had fewer branching points, the resulting global branching degree (which we shall denote as effective branching degree) would degenerate to values lower than  $r = 2$ .

Thus, as the molecule grows, serious problems may appear in maintaining the homogeneity as the molecule becomes denser in the external tiers, and a limit must logically exist, after which the homogeneity would be lost. In this work we have investigated that limit.

## The two-dimensional approach

### General considerations

This problem is certainly interesting, but the way to study it is not at all obvious. The model described previously by Meléndez-Hevia et al. (1993) (see above in Eqs. 1–4) proved to be particularly useful to study the optimization degree, but it cannot be used for this case because it is not

strictly a structural model. In effect, it describes full homogeneous molecules with any value of the external parameters whether they are or are not physically possible according to the structural constraints that operate during its synthesis. For example, as there must be at least four glucose residues between two branching points (see Fig. 3), the minimal length of a chain, leaving a tail of just one glucose residue, is  $g_c = 11$  for  $r = 2$ ,  $g_c = 16$  for  $r = 3$ , etc; and this is not taken into account by Eqs. 1–4. Here we want to study the influence of the physical constraints that operate on the building of a glycogen molecule, so we need a realistic model, which does not permit the making of physically impossible glycogens and which can describe accurately all, regular or irregular, possible structures.

The study of this problem is so complicated, as the cellular glycogen molecule is so big (see Fig. 4), that a procedure to simplify it is necessary. We have developed a two-dimensional (2D) approach as follows. Let us suppose that we are building a glycogen molecule drawing it on a plane paper in such a way that its chains cannot collide. The only difference with respect to what the cell does in 3D is that a stronger restriction of the available space is being made, but there is no other difference with respect to the structure of the cellular glycogen. The 2D system maintains the same properties as the original 3D one, because the reduction of space that occurs in each stage (tier) on molecular growth is a general fact, as in both dimensions the duplication of chains ( $r = 2$ ) does not come together with a duplication of available space in each tier. As there is less space in 2D than in 3D, such a reduction of space is greater in our model, so the same crowding effect occurs but in an earlier stage of growth. The 3D  $\rightarrow$  2D conversion maintains

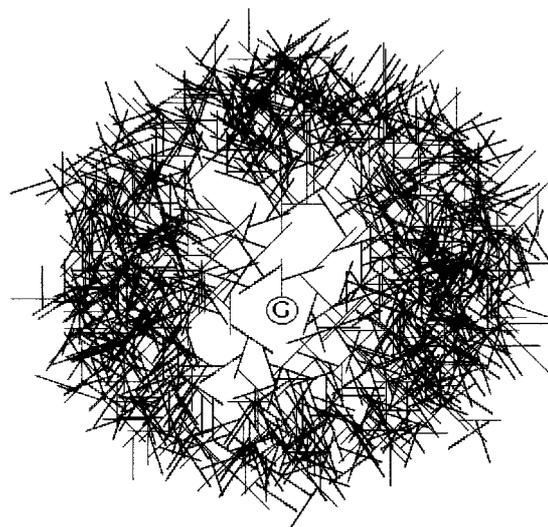


FIGURE 4 Full structure of the cellular glycogen. Picture drawn with a different version of the program Glycoplane, which allows the growth of all chains as occurs in the 3D molecule. This structure has the same parameters as the cell glycogen: branching degree  $r = 2$ , chain length  $g_c = 13$ , and number of tiers  $t = 12$ . This picture represents a molecule where some chains in external tiers have been set aside to show the low density in the inner zone. G, glycogenin, the primer peptide for glycogen biosynthesis.

the structural features of the molecule proportionally, and so the optimization function (Eq. 5), which is really a ratio between mass and space, transformed to 2D has also its maximum for  $g_c = 13$ .

### The computer program Glycoplane

Two-dimensional glycogens were built with the computer program Glycoplane, designed by us for the purpose of this work. It was made in Fortran 77, and it uses NAG libraries. A copy of the source file is available on request. Glycoplane generates and draws 2D structures made with straight lines branched in a very similar way as the real glycogen (see Figs. 5 and 6). Drawing operations are made filling a square matrix of  $700 \times 700$  pixels with sets of empty and full squares forming a set of branches, which represent the glucose chains. This is processed by 0/1 bits (0 means an empty pixel, and 1 a pixel full of material). Each glucose molecule is represented as a square of  $3 \times 3$  pixels, allowing a broad set of different angles for the branches that grow from each chain, giving 24 possible different directions (Fig. 5). Each helical glucose-polymer chain is represented as a straight line (Fig. 6).

The values of the parameters, namely, the branching degree ( $r$ ), the chain length ( $g_c$ ), and the number of tiers ( $t$ ) are introduced as data (external variables); Glycoplane fills the first chain, which is already the first tier, at the center of the matrix with the length specified by the  $g_c$  value, and each new chain is formed on every fifth residue. This distance between branches is the minimum possible, i.e., the distance that can be obtained in cellular glycogen under a high excess of the branching enzyme. Glycoplane thus creates a plane molecule simulating the cellular procedure, as in cellular conditions the branching enzyme is in excess of  $\sim 200$ -fold on synthase (cf. Meléndez et al., 1997); this procedure is repeated as many times as tiers are built.

Each time that a new chain is about to be built, Glycoplane checks how many residues can grow in each of the 24

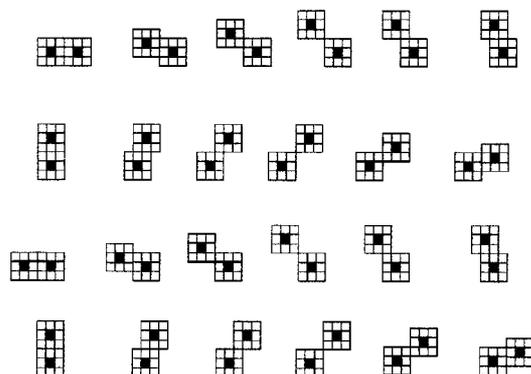


FIGURE 5 Glycosidic bonds used by Glycoplane. The 24 different bond angles between two glucoses. Each glucose is made as a square of  $3 \times 3$  pixels. The size of the squares was chosen to have a broad diversity of bond angles; they give enough possibilities to simulate the growth of the glucose polymer toward any direction in the glycogen molecule.

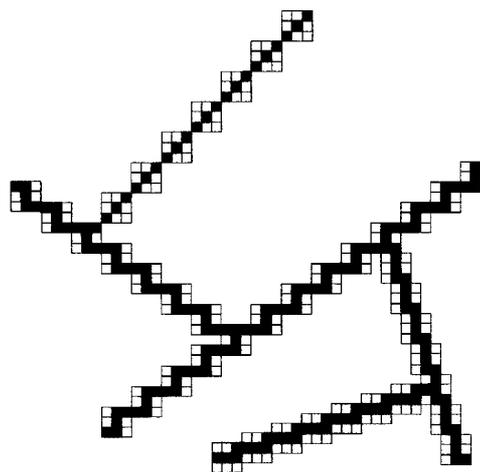


FIGURE 6 Detail of glycogen built by Glycoplane. Example of a structure made by the computer program Glycoplane. Each glucose residue is represented as a square of  $3 \times 3$  pixels, and a glucose polymer chain is represented as a straight line made up by a linear succession of squares attached according to one defined angle (see Fig. 5). The glycogen molecule drawn by the program has a tree-like shape, the branches of which are the lines formed by the central squares of each monomer, marked in black. Some examples of glycogens built by Glycoplane can be seen in Fig. 9.

possible directions (see Fig. 5) and eliminates those that would grow toward the inner areas, as it is not physically possible that synthase works progressing toward the core of the molecule. The procedure to make this elimination is to discard the growth of any chain with a distance between its end and the center of the molecule that is less than the theoretical radius of another belonging to two preceding tiers,  $R(t-2)$ ; this method generates a molecule with a more circular shape than that obtained by using  $R(t-1)$  as a reference point. Angles that do not allow the growth of at least seven residues are also discarded; this simulates the constraint imposed by the branching enzyme mechanism (Fig. 3).

Once the nonpermitted angles have been eliminated, Glycoplane selects one randomly among those that permit the longest length. This represents the freedom that the chains have in the real molecule to rotate to be able to take the direction that permits the growth with the full length. However, it does not impose that the space for a full chain (the input value for  $g_c$ ) must be available to start the building of a new chain, so shorter chains can be built, provided they have at least seven residues. If the available space does not permit the growth of chains with seven or more residues, then no chain is built, leaving inner chains with a lower branching degree. When a given increment  $[dx(i), dy(i)]$  (angle of the new chain) is chosen, all of the glucoses in the chain are drawn with the same increment as it really means a given angle of growth; this means that all chains are straight lines.

Under an input set of values of  $g_c$ ,  $r$  and  $t$ , Glycoplane builds a molecule with as many tiers as requested, growing only chains that have room to grow, the chains being shorter when there is no room for the full ones. Thus, the input

values of  $g_c$  and  $r$  are actually the maximum they can be. For this reason, we find chains with shorter length than the input value of  $g_c$ , but not longer, as well as inner chains with fewer branches than the given value of  $r$ .

### Conversion of variables from 3D to 2D

Let us now calculate the optimization function of the 2D glycogen by means of applying a general criterion of three dimensions (3D) to two dimensions (2D). In a body built with a homogeneous material, mass is directly related to size (volume in our 3D world and surface in a 2D world). Let us denote  $M_{3D}$  as the real mass we measure in the 3D space and  $M_{2D}$  as the mass the structure would have if the object were converted into a 2D structure. Denoting length as  $L$ , we can express this relationship as follows:

$$M_{3D} \sim L^3; \quad M_{2D} \sim L^2 \quad (6)$$

We can thus derive the following expression between the real mass ( $M_{3D}$ ) and the resulting mass from the 3D  $\rightarrow$  2D transformation ( $M_{2D}$ ) as follows:

$$M_{2D} \sim M_{3D}^{2/3} \quad (7)$$

This expression gives us the value of the new mass in 2D as a function of the real mass that we can measure in our 3D world. The exponent of this power function is a fraction with a denominator that is the dimension in which the real magnitude is embedded, and the numerator is the new dimension that our structure has after the transformation. The same reasoning can be made for the 3D  $\rightarrow$  2D conversion of space; thus, volume,  $V_{3D} = L^3$  in 3D is converted into  $V_{3D}^{2/3} = (L^3)^{2/3} = L^2$  (surface) in 2D; surface ( $L^2$ ) in 3D is converted into  $L^{4/3}$  in 2D, and length ( $L$ ) in 3D is converted into  $L^{2/3}$  in 2D.

The problem at which this work is aimed must be studied by exploring how the structural homogeneity may degenerate on increasing its density.

The optimization function (Eq. 5) defined for the real 3D cellular glycogen is a function of magnitude capable of suffering this 3D  $\rightarrow$  2D conversion, and thus, these transformations also affect it. Taking into account the transformations explained above, the optimization function is thus converted into

$$(f_{opt})_{2D} = \frac{C_A (G_T G_{PT})^{2/3}}{S_C}, \quad (8)$$

where  $S_C$  (surface of the circle) is here substituting for the volume of the sphere  $V_S$ . Equation 8 is plotted in Fig. 7. This function has also the maximum for  $g_c = 13$ , the same value as in the 3D function (Eq. 5). This is logical because our model is in fact a device that magnifies the space restriction that operates during the molecular synthesis by means of reducing the available space. The 2D glycogen molecules are subject to the same space constraint effect that happens in 3D, the only difference being that the crowding occurs earlier in 2D. This is what allows us to

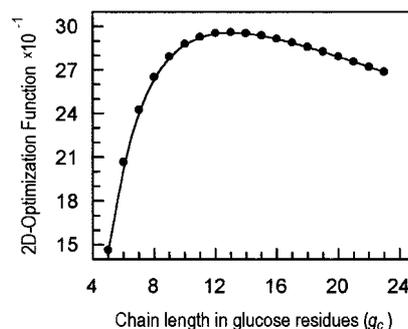


FIGURE 7 Optimization function of the 2D glycogen. Plot of the optimization function of plane glycogen structure (Eq. 8), according to the value of the chain length ( $g_c$ ). The function shows its maximum at  $g_c = 13$  as in the 3D glycogen.

work with smaller molecules, which can be more easily studied.

Let us see how the change of dimension affects the size of the molecule. It is obvious that a 2D glycogen will have fewer tiers than a 3D one, so what is the growth limit in the 2D glycogen? We assume, as is mentioned above, that this limit is determined in 3D when the percentage of space occupied by glucose reaches a certain value. We shall demonstrate now that the percentage of space occupied by glucose (a dimensionless magnitude) is independent of the dimension in which the structure considered is embedded.

Let us now imagine that we have a hollow cylinder of external radius  $R$ , internal radius  $r$ , and length  $h$ , the thickness of the casing being  $(R - r)$ . Let  $V$  be the total volume of the cylinder, and  $V'$  the volume of the hollow; so the volume of the casing  $v$  is

$$v = V - V' = \pi h (R^2 - r^2), \quad (9)$$

which can be expressed as a function of the total volume of the cylinder by  $v = pV$  being the fraction of volume that this casing occupies in the total volume of the cylinder:

$$p = \frac{v}{V} = \frac{\pi h (R^2 - r^2)}{\pi R^2 h} = 1 - \left(\frac{r}{R}\right)^2 \quad (10)$$

Let us imagine that we compress the cylinder reducing the length  $h$  without changing  $R$  or  $r$ . The fraction of the total volume of the casing,  $p$ , is unchanged at any point of the process as it does not depend on  $h$ . In the limit of that reduction, when  $h \rightarrow 0$ , the cylinder becomes a circle and the casing becomes a circular crown, as volume becomes surface, but  $p$  (dimensionless) is unchanged as it is independent from  $h$ . This can be generalized to any dimensionless magnitude, and thus, a given percentage of volume in the original object in 3D remains at the same value when the object is reduced to 2D. Therefore, if in the cellular 3D glycogen, 26% of space in the last 12th tier is occupied by glucose, determining the most permitted crowding, then the same value (26%) is the limit in the 2D-model glycogen. As we show below, this limit occurs at the sixth tier in the plane glycogen represented by our model.

### Space occupied by glucose

The percentage of space occupied by glucose in each tier in 2D was calculated by analogy as was done in 3D (Meléndez-Hevia et al., 1993). In the cellular 3D world, the volume of a glucose molecule,  $V_g$  (Van der Waals volume), is  $0.113 \text{ nm}^3$ ; the radius of the glycogen molecule with  $t$  tiers and a chain length of  $g_c$  glucose residues, considered as a sphere is

$$R_S (\text{nm}) = t \left( 0.24 \frac{g_c}{2} + 0.35 \right) \quad (12)$$

The amount of glucose in a given tier  $t$  is

$$glc(t) = g_c \frac{r^t - r^{t-1}}{r - 1}, \quad (13)$$

where  $r$  is the branching degree. According to these calculations, the volume occupied by glucose in the 12th tier is 26%.

A similar calculation can now be made for our 2D model as follows. Each glucose residue occupies in our model a square of  $9 (3 \times 3)$  pixels. The radius of the molecule can also be expressed in pixels. Equation 12 applies here as

$$R_S (\text{pixels}) = t \frac{3g_c}{2} + 3(t - 1) \quad (14)$$

Thus, we now calculate surfaces instead of volumes. Equation 13 applies here without change, as the amount of glucose molecules in each tier depends only on the branching degree and the chain length, it being independent of the dimension of the model.

### Measure of structural homogeneity

On growing the molecule beyond a certain limit, it loses structural homogeneity, as the branching degree and the chain length degenerate in the last tiers (see results below).

These effects were quantified as follows:

$$\% \text{ loss of } r = \frac{r_{\text{input}} - \overline{r_{\text{ef}}}}{r_{\text{input}}} \times 100, \quad (15)$$

where  $\overline{r_{\text{ef}}}$  is the mean of the effective branching degree of the glycogens built by Glycoplane in the four different simulations carried out for each case, and has been calculated as

$$r_{\text{ef}} = \frac{1}{t_{\text{input}} - 1} \cdot \sum_{t_i=2}^{t_{\text{input}}} \frac{\text{number of chains in } t_i}{\text{number of chains in } (t_i - 1)} \quad (16)$$

The percentage of loss of  $g_c$  was calculated in an analogous way. Table 1 shows how the built structures degenerate, losing homogeneity, which is measured with these two magnitudes described above. It also shows that the degeneration of the branching degree is the most representative magnitude to quantify the structure degeneration.

### Two-dimensional glycogen structures

As a consequence of its branched structure, growth of the glycogen molecule promotes chain crowding in the last tiers. If all chains grow in each tier making a full glycogen (if the effective branching degree is maintained at the same value without degeneration), then the percentage of occupied volume increases exponentially, reducing dramatically the available space that the enzymes need to build the molecule. According to Madsen and Cori's hypothesis, when this crowding reaches a limit of minimal free space, the growth of the molecule must be stopped.

Fig. 8 shows the percentage of space occupied by glucose in the different tiers of the three possible 2D glycogens (with  $r = 2, 3,$  and  $4,$  respectively) in two cases: 1) full glycogens, calculated with the former model (Eqs. 1–4) transformed for 2D (the physical constraints have not been taken into account there, so every tier is complete) and 2)

**TABLE 1** Degeneration of branching degree  $r$  and chain length  $g_c$

| Tier $t$ | $r_{\text{max}} = 2$ | $g_{c \text{ max}} = 13$ | $r_{\text{max}} = 3$ | $g_{c \text{ max}} = 17$ | $r_{\text{max}} = 4$ | $g_{c \text{ max}} = 22$ |
|----------|----------------------|--------------------------|----------------------|--------------------------|----------------------|--------------------------|
|          | % loss of $r$        | % loss of $g_c$          | % loss of $r$        | % loss of $g_c$          | % loss of $r$        | % loss of $g_c$          |
| 1        | 0                    | 0                        | 0                    | 0                        | 0                    | 0                        |
| 2        | 0                    | 0                        | 0                    | 0                        | 0                    | 0                        |
| 3        | 0                    | 0                        | 0                    | $1.80 \pm 2.02$          | $8.75 \pm 5.45$      | $1.52 \pm 2.00$          |
| 4        | 0                    | 0                        | $8.15 \pm 4.12$      | $4.97 \pm 1.11$          | $27.60 \pm 3.22$     | $9.24 \pm 2.45$          |
| 5        | $5.00 \pm 2.34$      | $0.25 \pm 0.32$          | $33.88 \pm 3.87$     | $9.02 \pm 2.24$          | $55.68 \pm 1.51$     | $18.31 \pm 1.58$         |
| 6        | $8.65 \pm 2.06$      | $2.21 \pm 0.58$          | $52.60 \pm 3.24$     | $8.82 \pm 0.92$          | $65.78 \pm 0.49$     | $13.09 \pm 0.67$         |
| 7        | $26.49 \pm 2.24$     | $2.44 \pm 0.89$          | $57.04 \pm 1.21$     | $10.86 \pm 1.32$         |                      |                          |
| 8        | $34.59 \pm 2.23$     | $5.51 \pm 1.01$          |                      |                          |                      |                          |
| 9        | $40.32 \pm 2.78$     | $3.82 \pm 0.56$          |                      |                          |                      |                          |

Effective branching degree ( $r$ ) and chain length ( $g_c$ ), in the different simulations as the number of tiers  $t$  increases, have been calculated with Eqs. 15 and 16 for  $r$  and in an analogous way for  $g_c$  and show the degeneration of these variables as the molecule grows. The degeneration of the branching degree is much more dramatic than that of the chain length. This is due to the information that these variables contain. Whereas  $r$  has the information about how many chains could grow with respect to those that would have grown if the molecule were homogeneous,  $g_c$  has information only about the length of those that could grow. Thus, the degeneration of  $r$  is the best way to quantify the loss of structural homogeneity.

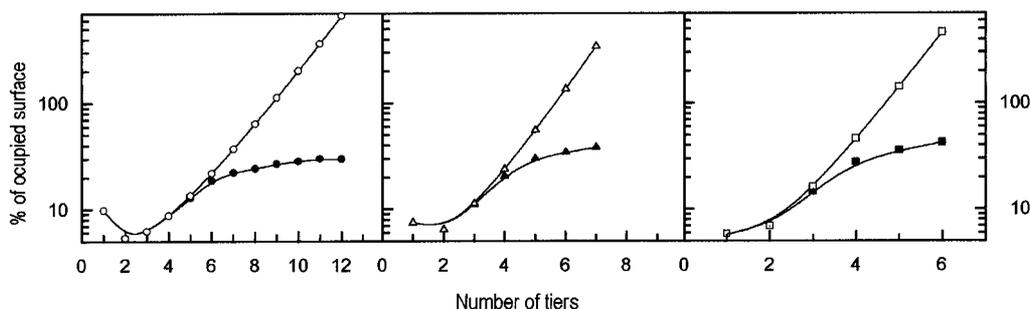


FIGURE 8 Space occupied by glucose in the glycogen molecule. Percentage of surface occupied by glucose in each tier of different glycogens according to their branching degrees:  $r = 2, 3,$  and  $4$ . Open symbols represent data from glycogens calculated with the former model (Eqs. 1–4); they represent hypothetical glycogens with all their tiers full. The branching degrees always persist in every tier. Solid symbols represent data from glycogens built with the computer program Glycoplane; they are realistic, as the physical constraints have been taken into account to build them. Thus, the occupied surface never surpasses a low percentage of the total space. Until the points where the curves diverge, all glycogens built have a good homogeneity, as their percentages of full chains are, respectively,  $92.86 \pm 0.88$  ( $t = 6, r = 2$ ),  $86.28 \pm 2.94$  ( $t = 4, r = 3$ ), and  $94.79 \pm 3.12$  ( $t = 3, r = 4$ ). Beyond these points the structural homogeneity of each realistic glycogen (*solid symbols*) degenerates as the percentages of occupied surface come apart from the curves of hypothetical full molecules (*open symbols*). The percentage of the surface occupied by glucose obviously exceeds 100% after a certain tier, as these calculations correspond to hypothetical glycogens without taking into account physical constraints. Such nonrealistic glycogens are useful as reference points to compare with the physically possible ones.

realistic glycogens created with Glycoplane, which takes into account the physical constraints (see Fig. 9). Full glycogens show an exponential ratio (linear in a logarithmic scale) between the number of tiers and the surface that would be occupied by glucose if all tiers were full. This means a severe space constraint on growing the molecule. Thus, in realistic glycogens built with Glycoplane, as density in the last tiers increases, the percentage of space occupied by glucose increases slowly, going toward a constant value, as several branches do not grow, leading to a degeneration of the branching degree. It is interesting to see that until the sixth tier the glycogen built by our model is equal to the full one. This means that the building of such a full glycogen is physically possible, whereas glycogens with more tiers cannot maintain a homogeneous structure.

In full 2D glycogens with  $r = 2$ , the surface occupied by glucose is 21.93% in the sixth tier and 36.97% in the seventh tier. We have demonstrated that the 3D  $\rightarrow$  2D conversion does not change the limit percentage of occupied space; that is, the permitted density limit is the same in 2D as in the cellular glycogen (26%). Thus, we can conclude that for a 2D glycogen with  $r = 2$  and  $g_C = 13$ , the density limit imposes that it cannot have more than six tiers, which is equivalent to the cellular molecule with 12 tiers. This molecule, drawn in Fig. 9 A, is practically full, its effective branching degree is very near to 2, and the space occupied by glucose is 21.9%. If the synthesis of the 2D glycogen molecule were forced to continue further under the same physical constraint (the space occupied by glucose cannot exceed the 26% (which is a realistic condition), a number of chains could not grow in the following tiers. Our approach allows us to see that the branching degree degenerates in these oversized molecules, and this effect is worsened quickly at higher  $t$  values (see Figure 10) leading to a highly inhomogeneous molecule with bad metabolic efficiency, as their value of the optimization function declines greatly under these conditions (see Figure 11).

Figures 10 and 12 show that the loss of structural homogeneity on growing the number of tiers is slow when  $r = 2$  and much faster for higher values, as  $r$  determines the degree of the exponential increase of the crowding (see Eq. 1). The value  $r = 2$  of the cellular glycogen thus produces the highest structural homogeneity of the molecule. These data also demonstrate that the number of tiers of the full molecule ( $t_{\max}$ ) is an optimizable variable as a certain limit tier exists beyond which the structural properties of the molecule degenerate.

### Concluding remarks

In this work we have demonstrated that the number of tiers of the glycogen molecule is an optimizable variable. The reason behind this fact is the structural homogeneity of the glycogen molecule and how the mechanism of its synthesis can account for its achievement. Our results show that the space constraints that operate during the synthesis of glycogen can determine structural irregularities in the glycogen molecule by means of the growth of incomplete tiers, damaging the metabolic function of glycogen. The degeneration of the branching degree is a good way to measure such a loss of homogeneity (see Figures 10 and 12 and Table 1).

Thus, our results show that the molecule has an optimal size, which is the maximum that maintains its structural homogeneity. This would be determined by the size of the synthesizing enzymes, which could not work once the last tier reaches a certain density. Thus, our results suggest that the size of the synthesizing enzymes has been optimized so that glycogen synthesis stops at the right point. We can arrive at this conclusion because the only restrictions that our computer program Glycoplane takes into account are those related to the space occupied by glucose molecules, not to the size of the enzymes.

Once we know that  $t_{\max}$  is an optimizable variable, the question is whether cellular glycogen has achieved this

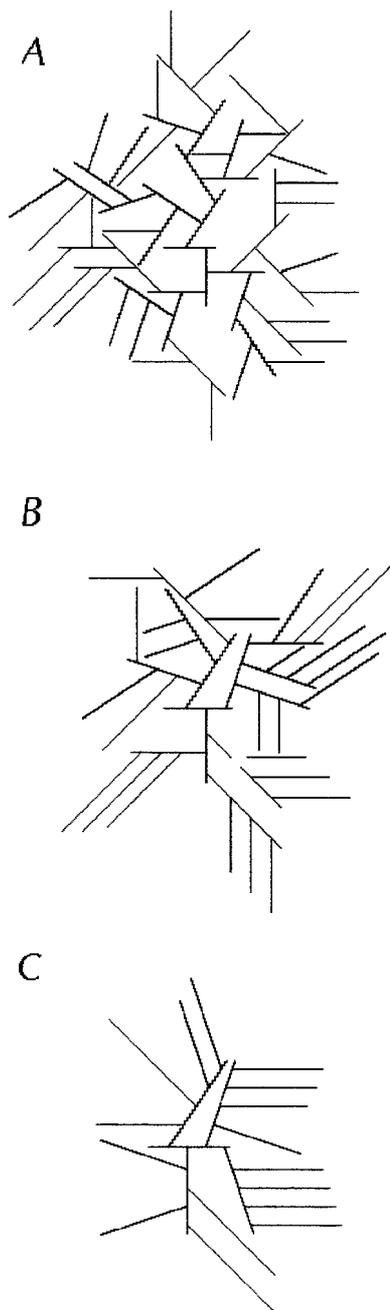


FIGURE 9 Two-dimensional glycogens. Three plane glycogens drawn by the computer program Glycoplane with different branching degree ( $r = 2, 3,$  and  $4$ ). In each case, the chain length must be in accordance with the branching degree to allow  $r$  branching points on each internal chain, as at least four glucose residues must always be between every two branches. It can be seen that the molecule with  $r = 2$  shows the higher structural homogeneity.

maximum. In other words, is  $t = 12$  the optimal number of tiers of a glycogen molecule? In principle, seeing only the percentages that full tiers would occupy in 2D (37% for  $t = 7$ ; 64% for  $t = 8$ ; see Fig. 8), one could think that these tiers could also have grown (see Fig. 9), but our results show that  $t = 6$  is the maximal number of tiers that a 2D molecule can have to maintain its branching degree ( $r = 2$ ), as Fig. 8

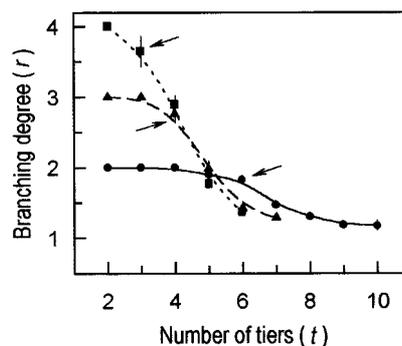


FIGURE 10 Degeneration of the branching degree with the number of tiers. Data of three plane glycogens with branching degree  $r = 2$  (●),  $r = 3$  (▲), and  $r = 4$  (■). If the molecules are forced to grow further from their normal limit (marked with the *arrows*), incompletely filled tiers are built (the branching degree degenerates). These glycogens have a poor structural homogeneity and cannot play their metabolic role well. Values of  $g_c$  (maximal chain length) are  $g_c = 13$  for  $r = 2$ ,  $g_c = 17$  for  $r = 3$ , and  $g_c = 22$  for  $r = 4$ . Branching degree was empirically calculated by Eq. 16. Standard deviations were usually very small, and so they can be seen only in some points. The degeneration is slow with  $r = 2$  and much faster for higher  $r$  values.

shows. Thanks to our structural model, we have been able to prove that a percentage of occupied space under 100% is not a sufficient condition for this tier to be able to grow fully.

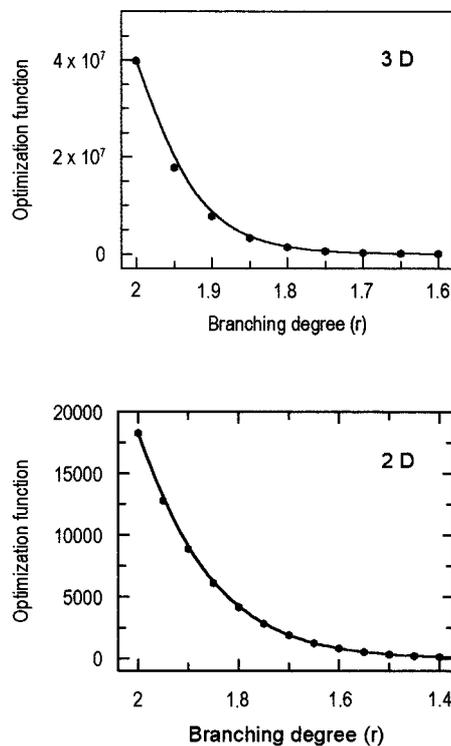


FIGURE 11 Decline of the optimization function with the degeneration of the branching degree. The chain crowding in the last tiers of glycogen can cause nongrowth of some chains, losing structural homogeneity. This figure shows that such a glycogen is poorly optimized and cannot account for a good metabolic function. Data were calculated for 3D and 2D molecules.

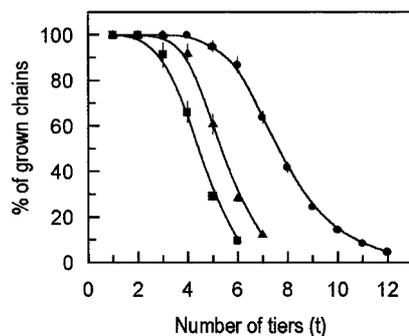


FIGURE 12 Percentage of chains that grow in each tier. On growing the molecule the growth of new chains after a given point is restricted by space constraints. If the molecule is then forced to grow beyond that normal limit, some chains cannot grow, so the percentage of grown chains (in relation to the total number of chains that would grow if there were no space restriction) decreases. The figure represents this effect in three kinds of glycogen with different initial branching degree and chain length:  $r = 2$ ,  $g_c = 13$  (●);  $r = 3$ ,  $g_c = 17$  (▲); and  $r = 4$ ,  $g_c = 22$  (■). This effect is less dramatic with  $r = 2$ .

Thus, it is very reasonable to expect that the same happens in 3D and, consequently, although the 13th full tier would occupy 62% of the volume, the building of all of these chains would also be impossible. We conclude that  $t = 12$  is the maximal number of tiers that a glycogen molecule can have to have all its tiers full.

Structural homogeneity is a structural feature of the glycogen molecule that had not been taken into account in previous studies but that has an obvious high fitness value to guarantee a good functioning of the glycogen molecule. Thus, it should also be considered in the whole optimization target of glycogen structure. In general, our results presented in Figs. 8, 10, and 12 and Table 1 show that the glycogen molecule, with the parameters that operate in cellular metabolism, has the highest value of the optimization function with also a high degree of structural homogeneity. Higher values of the branching degree would lead to a quicker loss of this property, which confirms the previous results about the optimization of  $r$  (Meléndez-Hevia et al., 1993; Meléndez et al., 1997).

We should like to emphasize the role of the 2D approach in this work. The high complexity of the structure forced us to work with a reduced version of it; the problem was whether such a reduction was possible and whether the study at such level could give us enough information. The aim of this work was to study the consequences of space restrictions that operate in glycogen synthesis and their consequences on the structure and metabolic effectiveness of the product. Then, the 2D model permitted us to have a controllable space (a surface) where the position of each chain could be accurately known.

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