Optimization of molecular design in the evolution of metabolism: the glycogen molecule

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The animal glycogen molecule has to be designed in accordance with its metabolic function as a very effective fuel store allowing quick release of large amounts of glucose. In addition, the design should account for a high capacity of glucose storage in the least possible space. We have studied the optimization of these variables by means of a mathematical model of the glycogen molecule. Our results demonstrate that the structure is optimized to maximize (a) the total glucose stored in the smallest possible volume, (b) the proportion of it that can be directly released by

phosphorylase before any debranching occurs, and (c) the number of non-reducing ends (points of attack for phosphorylase), which maximizes the speed of fuel release. The optimization of these four variables is achieved with appropriate values for two key parameters in glycogen design: the degree of branching and the length of the chains. The optimal values of these two parameters are precisely those found in cellular glycogen.

INTRODUCTION

One of the most characteristic features of biological evolution is its role as an optimization process, including optimization of cellular metabolism, the chemical machinery behind every biological function. An example of this is the optimization of the pentose phosphate cycle (Meléndez-Hevia and Isidoro, 1985; Meléndez-Hevia and Torres, 1988; Meléndez-Hevia, 1990). These results suggested that other cases of optimization of metabolic design could be found, and therefore the aim of the present work was to investigate the optimization of molecular design of the structure of glycogen.

The fuel-storage role of glycogen in cellular metabolism is well known [see, e.g., Stryer (1988), Voet and Voet (1990) and Harris (1992)]. The function, and consequently the molecular design, of such a material is not simple. The main function of skeletalmuscle glycogen is to be the source of glucose for anaerobic glycolysis. Muscle anaerobic glycolysis is not only a process to produce ATP without an oxygen supply; it is primarily a rapid metabolic source of ATP, which makes rapid macroscopic motion possible. It is a relatively short pathway, making ATP without the Krebs cycle and respiratory chain, and therefore it needs less time to reach the steady state (Easterby, 1981; Torres et al., 1991). The amount of glucose spent during anaerobic glycolysis (about 17 times more than aerobic metabolism of glucose for supplying the same amount of ATP) requires a very large glucose store which should obviously be as near as possible to contractile myofibrils. Despite its poor energy yield, the role of anaerobic glycolysis is clear: it provides good support of quick movements.

Glycogen must therefore have an appropriate design for this role. The first important feature is that it is a polymer; this is a very efficient way of storing a large amount of cytoplasmic glucose without causing a significant increase in osmolarity. It is

well known that the cytoplasm is near the limit of its solvent capacity (Atkinson, 1969; see also Ovádi, 1991). It has been calculated that the total glucose stored in liver cells as glycogen is equivalent to 400 mM, whereas the concentration of glycogen is only 0.01 μ M (Harris, 1992). Branching provides many points of attack for phosphorylase, allowing more glucose to be released simultaneously. The number of non-reducing ends in a glycogen particle is, for spatial reasons, much greater than the number of active sites of phosphorylase that can be simultaneously attacking the molecule; there are actually about 20-25 phosphorylase tetramers, each with a molecular mass of 400 kDa, in one β particle of glycogen, which has a diameter of 40 nm (Madsen and Cori, 1958); such a particle has about 55000 glucose residues (molecular mass 10⁷ Da) and about 2100 non-reducing ends (Goldsmith et al., 1982). However, the number of non-reducing ends must be much higher than the number of active sites of phosphorylase attached to the molecule; a high number of nonreducing ends enhances the capacity of the enzyme to bind glycogen, as this is equivalent to increasing the concentration of the substrate. However, glycogen degradation by phosphorylase is not a simple mechanism, there being important geometrical problems involved in adjusting the structure of the enzyme to that of the glycogen. Phosphorylase has a given tetrameric structure, which imposes a spatial relationship on its glycogenbinding sites. Owing to the polymeric structure of glycogen, the (1-4) glycosidic bonds which are attacked by a single molecule of phosphorylase do not have the freedom to adopt the spatial orientation required by phosphorylase. As phosphorylase progresses along one chain using one active site, the other active sites might lose their bonds to the substrate. However, this problem could be decreased if (1) the external branches (exposed to the action of phosphorylase) are long enough, as long chains allow more flexibility, and (2) there is a large enough number of such chains in order to guarantee that an active site of phosphorylase

Abbreviations used: $C_{\rm A}$, number of A-chains; $C_{\rm T}$, total number of chains; $g_{\rm c}$, number of glucose residues in each chain; $G_{\rm PC}$, number of glucose residues available for phosphorylase in each A-chain; $G_{\rm PT}$, total amount of glucose available to phosphorylase; $G_{\rm T}$, total amount of glucose stored in the glycogen molecule; $L_{\rm t}$, length of each tier; r, degree of branching; $R_{\rm s}$, radius of the glycogen sphere; t, number of tiers; $V_{\rm s}$, volume of the glycogen sphere.

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made empty by losing its chain can be immediately filled again by another conveniently placed chain.

The kinetic design of glycolysis and glycogen metabolism agrees well with the aim of quick release of large amounts of glucose 1-phosphate; it has been demonstrated by several groups that the availability of phosphorylated glucose is the most critical variable in controlling glycolytic flux (see Helmreich and Cori, 1965; Rapoport et al., 1974; Aragón et al., 1980; Fell, 1984; Meléndez-Hevia et al., 1984, 1992; Torres et al., 1986, 1988). On the other hand, the regulatory mechanism that triggers the release of glucose 1-phosphate from glycogen has been shown to be extremely efficient and quick. Cárdenas and Cornish-Bowden (1989) have calculated that, under a given set of conditions, an enzyme cascade could generate a highly sensitive response, equivalent to a Hill coefficient of 800. This regulatory mechanism is really a switch; no single allosteric or Michaelis mechanism could ever account for such a response. Furthermore, these results show that such an activation, in addition to being very efficient, is also extremely rapid. The effect of amplification in such a cascade mechanism agrees with the necessary amount of enzyme involved; phosphorylase is enormously abundant in skeletal muscle [2% of the total soluble protein (Ryman and Whelan, 1971)]. Furthermore, phosphorylase and glycolytic enzymes are much more active in white muscles (anaerobic) than in aerobic red muscles (Opie and Newsholme, 1967; Newsholme and Start, 1973; Banks et al., 1976). Finally, glycogen depletion seems to be the trigger for the chain of events responsible for muscle fatigue (Bertocci et al., 1992).

The efficiency of relating animal macroscopic behaviour to glycogen metabolism depends on a good design of the glycogen molecule. The role of this material in metabolism requires a design that maximizes the capacity for storing a very large amount of glucose in the least possible volume, and that, under attack by the appropriate enzymes, allows as much glucose as possible to be released quickly. The design of such a molecule depends on the value of certain parameters, namely the degree of branching and the length of the chains. The question is: are the values of these parameters produced by evolution the most appropriate for an optimized design of the glycogen molecule?

We present here a mathematical model that describes the structure of glycogen and allows us to calculate the values of its parameters that optimize the variables mentioned above. Our results demonstrate that the structure of the glycogen molecule has an optimized design for maximizing (a) the total glucose stored in the smallest volume, (b) the amount of it that can be directly released by phosphorylase, before any debranching, and (c) the number of non-reducing ends (points of attack for phosphorylase), which maximizes the speed of fuel release. The optimization of these four variables is achieved with appropriate values of two key parameters in glycogen design: the degree of branching and the length of the chains. The values of these two parameters are precisely those of cellular glycogen.

GLYCOGEN STRUCTURE AND MATHEMATICAL MODEL

Glycogen structure

Although several details of the fine structure of glycogen are still unknown, the available information allows us to make a model suitable for the purpose of this work. The model of Whelan [Gunja-Smith et al. (1970); see also Gunja-Smith et al. (1971)] mainly derived from data on enzymic degradation of glycogen is generally accepted (Goldsmith et al., 1982; Bullivant et al., 1983). According to these results, the main features of glycogen structure can be described as follows (see Figure 1). The glycogen

molecule is formed by two different kinds of chain: B-chains, which are branched, and A-chains, which are not. The branching of the B-chains is uniformly distributed (with degree of branching equal to 2), so every B-chain has two branches on it, creating further A- or B-chains. There are four glucose residues between branches and a tail after the second branch in the B-chains. A-

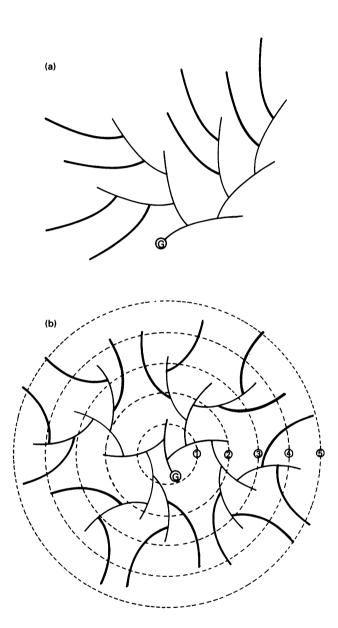


Figure 1 Scheme showing the structure of the glycogen molecule as stated in Whelan's model (Gunja-Smith et al., 1970, 1971; see also Goldsmith et al., 1982)

There are two kinds of chains: A and B. A-chains are not branched, having only (1—4) bonds. B-chains are branched, each of them with two branching points [(1—6) bonds] which create either B- or A-chains. The molecule is spherical and organized into concentric tiers. Every B-chain is in the inner tiers, and every A-chain is within the outer tier. The degree of branching (r) is 2; therefore, each tier has twice the number of chains as the previous one. Each A- or B-chain has 12—14 glucose residues ($g_c \approx 13$) and there are 12 tiers (t = 12) in the molecule, which means about 53 000 glucose residues. Glycogenin, a small protein (G in the Figure) in the core of the glycogen molecule, plays the role of primer in glycogen synthesis. The whole molecule is called: a β -particle of glycogen; it has a diameter of 21 nm and a molecular mass of 10^7 Da. (a) Extended structure to show the branching structure; (b) a more realistic drawing showing the disposition of the successive branches forming concentric tiers (numbered circles). Both schemes show a simplified molecule with only four tiers in (a) and five in (b): A-chains, in the outermost tier, are more thickly drawn.

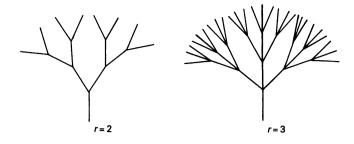


Figure 2 A very simplified scheme for the structure of glycogen, showing the properties that derive from the degree of branching (r=2 in cellular glycogen)

Greater values of r give an extremely dense molecule with a poor capacity for storing glucose as it can contain few tiers (see Table 1). In order to simplify the Figure, the scheme has been drawn locating every branch starting from the same point in each chain, and eliminating each end tail. The real structure of glycogen can be seen in Figure 1.

and B-chains are of uniform length, both mean value 13 glucose residues. Phosphorylase can only work on A-chains, as the tail of the B-chains is too short (about 4 glucose residues, which is at the limit of phosphorylase action). The glycogen molecule is spherical and organized into concentric tiers; every tier has the same length (1.9 nm). There are 12 tiers in a β -particle, with a total radius of 21 nm. Every A-chain is in the most external tier (number 12). As a consequence of the degree of branching (r=2), the number of chains in any tier is twice that of the previous one, and the same number of chains as the summation of all other previous tiers [see below, eqns. (12-13)]. From these data it can be derived that there are the same number of A-chains (all of them in the last tier) as B-chains, and that the amount of glucose directly available to be released by phosphorylase is 34.6 % of the total molecule, independent of the size (number of tiers) of the particle.

This manner of molecular organization has a number of features of great interest for studying the optimization of molecular design (in addition to those pointed out above). In effect, the fractal structure (see Figures 1, 2 and 4) guarantees that a number of properties remain when one or more tiers are gone. However, even within this same structure, there are a number of parameters with values that could be different, namely (a) the chain length, (b) the degree of branching and (c) the number of tiers. Any value for these parameters that differed from those of cellular glycogen would give a molecule with a similar shape but different properties. As we can define precisely the function of the glycogen molecule, we can investigate whether. in animals, it is optimally designed to meet the energy needs and whether different values for its parameters would result in a more efficient molecule. Has the course of evolution provided the perfect design? The answers to these questions will provide a better understanding of biochemical evolution and the relationship between molecular structure and its physiological role.

We here analyse these questions by using a mathematical model which describes the structure of glycogen. The model is derived under a set of hypotheses according to the general features of the molecular structure given by Gunja-Smith et al. (1970) and Goldsmith et al. (1982) [see also Bullivant et al. (1983)]. The purpose of this study is to obtain values for the three parameters mentioned above, in order to obtain a molecular design that allows maximization of total glucose stored, glucose available to phosphorylase and number of A-chains (i.e. points of attack for phosphorylase) and minimization of the molecular

volume, and to compare these results with the parameters known for cellular glycogen.

Mathematical model

The structure of the glycogen molecule can be described by the following set of equations: let r be the degree of branching (number of branching points on each B-chain), and thus, the factor that multiplies the number of chains in a tier giving the number of chains in the next one. Let t be the number of tiers in the molecule. Then, the total number of chains (C_T) is:

$$C_{\rm T} = \sum_{t=1}^{t} r^{(t-1)} = \frac{1 - r^t}{1 - r} \tag{1}$$

and the number of chains in a given tier t,

$$C_{t_i} = r^{(t_i - 1)} \tag{2}$$

Every A-chain is in the most external tier. Thus eqn. (3) gives us the number of A-chains:

$$C_{\mathsf{A}} = r^{(t-1)} \tag{3}$$

Let g_c be the number of glucose residues in any A-chain. It is known that not all of these units can be released by phosphorylase. A logical physical limit exists to the ability of phosphorylase to digest any A-chain. This limit was empirically determined by Walker and Whelan (1960) as 4. There are clear steric reasons that can justify the value of this parameter, so we take it as fixed in our reasoning. So the number of glucose residues available for phosphorylase in each A-chain (G_{PC}) is:

$$G_{\rm PC} = g_{\rm c} - 4 \tag{4}$$

The total amount of glucose available to phosphorylase in the whole molecule (G_{PT}) is written as:

$$G_{\rm PT} = C_{\rm A} G_{\rm PC} = C_{\rm A} (g_{\rm c} - 4)$$
 (5)

and the total glucose in the whole molecule (G_{τ}) :

$$G_{\mathbf{T}} = C_{\mathbf{T}} g_{\mathbf{c}} \tag{6}$$

which by using eqn. (1) gives:

$$G_{\rm T} = g_{\rm c} \sum_{i=1}^{t} r^{(i-1)} = g_{\rm c} \frac{1 - r^t}{1 - r} \tag{7}$$

According to Goldsmith et al. (1982), on average, a branch starts halfway up a chain, and it gains about 0.35 nm because of the (1-6) bond; in the same paper they give a length of 0.24 nm per glucose in a chain. This gives an effective length per tier (L_i) of

$$L_{\rm t}(\rm nm) = 0.12\,g_{\rm c} + 0.35\tag{8}$$

The molecule has a spherical shape; this is consistent with Whelan's model and experimental data from other groups (see, e.g., Madsen and Cori, 1958), including electron micrographs. The radius of the sphere with t tiers is:

$$R_s = L_t t$$

which applying eqn. (8) is:

$$R_{\rm s}({\rm nm}) = t(0.12g_{\rm c} + 0.35)$$

and the volume of the sphere is:

$$V_{\rm s}(\rm nm^3) = \frac{4}{3}\pi t^3 (0.12g_{\rm c} + 0.35)^3 \tag{9}$$

Thus, eqns. (3), (5), (7) and (9) allow us to calculate any of the variables of the glycogen molecule mentioned above as a function of r, t and g_c . We shall use them to find the values for these parameters that optimize the variables.

Table 1 Variation of the density of the glycogen molecule and the total amount of glucose available to phosphorylase (G_{PT}), without any debranching, with the degree of branching (r) and the number of tiers in the molecule (t) (see also Figure 2)

A spherical shape is assumed. Density is given in arbitrary units. G_{PT} was calculated assuming a chain length of 13 glucose residues and a stub of 4 residues (the limit for phosphorylase action in each chain); the values of these parameters involve no loss of generality for these conclusions. Underlined numbers correspond to the cellular glycogen (r=2); in columns for r>2 the values with density equivalent to cellular glycogen are emphasized. Note that a glycogen with r>2 would have very poor glucose-storage ability. These data were obtained by computer calculation of eqns. (2), (4), (5), (6) and (9). Total glucose stored is not shown.

t	<i>r</i> = 1		r = 2		r = 3		r = 4	
	Density	G_{PT}	Density	G_{PT}	Density	G _{PT}	Density	G _{PT}
3	11.6	9	27	36	50	81	81	144
4	7.9	9	30	72	79	243	169	576
5	5.7	9	36	144	139	729	392	2304
6	4.3	9	46	288	264	2187	988	9216
7	3.4	9	62	576	530	6561	2649	36864
8	2.7	9	87	1152	1118	19683	7444	147 456
9	2.2	9	127	2304	2 444	59049		
10	1.9	9	191	4608	5510	177 147		
11	1.6	9	294	9216	12733	531 441		
12	1.4	9	<u>463</u>	18432				
13	1.2	9	741	36864				
14	1.0	9	1206	73 728				

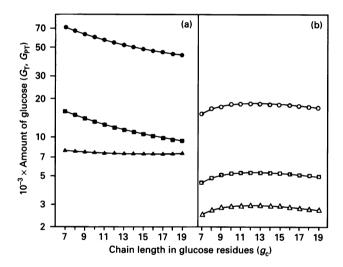


Figure 3 Glucose-storage capacity and number of glucose residues directly available to phosphorylase of different glycogen molecules, the design of which varies in degree of branching (r) and the chain length in glucose residues (g_c)

These values were calculated for glycogens with the same density as cellular glycogen (see also Table 1), by computer calculation of eqns. (3), (5), (7) and (9). (a) total glucose stored (G_7) ; (b) total glucose available to phosphorylase (G_{P7}) . looplus, \bigcap , r=2; \blacksquare , \bigcap , r=3; \blacktriangle , \triangle , r=4.

OPTIMIZATION OF GLYCOGEN MOLECULAR DESIGN

The degree of branching

The degree of branching in the glycogen molecule cannot be very high, as it would lead to an extremely dense molecule which would be useless for both phosphorylase action and the primary purpose of fuel storage. This fact is illustrated in Figure 2. The relationship between the degree of branching, the density of the glycogen particle and the total amount of glucose available to phosphorylase is shown in Table 1. It can be seen that, if the

degree of branching were r = 3, then a molecule with the same density as cellular glycogen could only have seven tiers; such a molecule would be much less efficient than the cellular one: its capacity for storing glucose would be 27% of cellular glycogen. and glucose available to phosphorylase only 36% of cellular glycogen. These values decrease dramatically for larger values of r (e.g. for r = 4, a molecule with a similar density would have five tiers, 8% of stored glucose and 13% of glucose available to phosphorylase). Values shown in Table 1 were calculated for a chain length of 13 glucose residues; this does not involve any loss of generality for these conclusions. The total glucose stored and the quantity of it available to phosphorylase are presented in Figure 3 for glycogens with different chain lengths but the same glycogen density (r = 1 means no branching; therefore, only)values of r equal to or above 2 are considered). From the results shown in Table 2 and Figure 3, it is clear that a degree of branching of r = 2, whatever the values of the other parameters. maximizes both the glucose-storage capacity and the amount of glucose available to phosphorylase. This is precisely the degree of branching in cellular glycogen in Whelan's model, as stated by Gunja-Smith et al. (1970, 1971) and later confirmed by Goldsmith et al. (1982) and Bullivant et al. (1983) among others. It is interesting to note that when r = 2, then the number of chains in the most external tier (12) approximately equals the number of chains in all the inside tiers, as:

$$2^{t} \approx \sum_{i=1}^{t-1} 2^{i} = 2^{t} - 1 \tag{10}$$

The following relationship is also useful in estimating the relative number of A-chains:

$$C_{\mathrm{T}} = \frac{1 - C_{\mathrm{A}}r}{1 - r} \tag{11}$$

which for the particular case (r = 2) gives

$$C_{\mathbf{T}} = 2C_{\mathbf{A}} - 1 \tag{12}$$

and therefore

$$C_{\rm A} = \frac{C_{\rm T} + 1}{2} \tag{13}$$

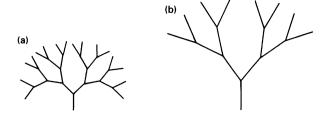


Figure 4 Scheme illustrating the problem of optimization for determining the most appropriate chain length in the design of the glycogen molecule

The different properties of two glycogen designs with the same degree of branching (r=2) but different chain lengths are shown. The design in (a) has short chains and the design in (b) has long chains. The Figure shows two glycogens with the same amount of stored glucose (the same amount of ink was spent on each one). (a) is a dense molecule, in which the same amount of stored glucose occupies less space, and has more points of attack for phosphorylase. However, the chains are short and so the enzyme can act only to a limited degree on each. (b) is a less-dense design, which has a bigger volume for the same amount of stored glucose; the length of its chains allows a continuous action of phosphorylase for a longer time. However, there are fewer points of attack.

The chain length

The above results show that r must equal 2 to guarantee the maximum effectiveness of glycogen as a large rapidly released store of glucose. After fixing this value, chain length is the other key parameter in the molecular design. Figure 4 shows two glycogen molecules which have the same amount of stored glucose, but different chain lengths; they are short in Figure 4(a) and long in Figure 4(b). It can be seen that a number of properties are derived from the value of this parameter; for example, for a given total amount of glucose, the design in Figure 4(a) occupies less space and has more A-chains (more tiers), but they are longer in the design in Figure 4(b) and there are more glucose units available to phosphorylase.

As stated above, the four properties that an optimized molecule of glycogen should have are: (a) maximum points for phosphorylase attack; (b) maximum stored glucose; (c) maximum glucose residues directly available to phosphorylase with no previous debranching; and (d) minimum volume. Figure 5 shows the variation in these properties with variation in g_a. It is clear that all these properties must be optimized and that this is a typical case of multiobjective optimization. Each of these properties can be expressed by a given variable, all of them related by the equations of the model described here: A-chains $(C_{\scriptscriptstyle A})$ are the points of phosphorylase attack, $G_{\scriptscriptstyle {
m T}}$ is the total glucose stored in the molecule, $G_{\rm PT}$ is the total glucose available to phosphorylase and V_s is the volume of the molecule. The relationships among these variables are given by eqns. (3), (5), (7) and (9). In accordance with previous reasoning, the aim now is to find the values of t and g_e that maximize C_A , G_T and G_{PT} and that minimize V_s , i.e. to maximize the function:

$$f = \frac{G_{\rm T}C_{\rm A}G_{\rm PT}}{V_{\rm c}} \tag{14}$$

 $C_{\rm A}$, $G_{\rm T}$, $G_{\rm PT}$ and $V_{\rm s}$ being related by eqns. (3), (5), (7) and (9). We know that r must equal 2. Therefore eqn. (14) can be written as:

$$f = K \frac{g_{c}(g_{c} - 4)}{(0.12g_{c} + 0.35)^{3}}$$
 (15)

with

$$K = \frac{(6C_{\rm A} - 3)C_{\rm A}^2}{4\pi t^3} = \frac{[6 \times 2^{(3t-3)}] - [3 \times 2^{(2t-2)}]}{4\pi t^3}$$
 (16)

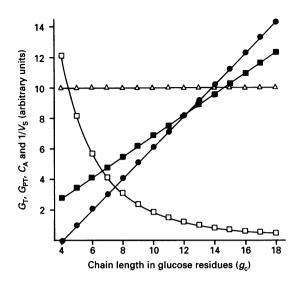


Figure 5 Variation in the four variables to be optimized in the design of glycogen as a function of the chain length in glucose residues (g_{*})

■. Total glucose stored (G_7) ; ●, total glucose available to phosphorylase attack (G_{P7}) ; △, points of attack for phosphorylase, as number of A-chains (C_A) ; \Box , 1/volume of the molecule $(1/V_s)$. These data were calculated by computer calculation of eqns. (3), (5), (7) and (9).

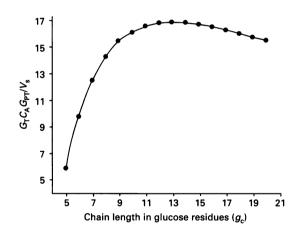


Figure 6 Optimization of the chain length (g_*) in the glycogen molecule in order to maximize the glucose-storage capacity in the least possible volume, the total amount of glucose that can be released by phosphorylase before any debranching occurs and the points of attack for the enzyme

The graph represents the value of $f=G_{\rm T}C_{\rm A}G_{\rm PT}/V_{\rm S}$ [eqn. (14)]. These results were obtained by computer calculation of eqn. (15). They show that a value close to 13 (12.93) is the value of $g_{\rm c}$ that maximizes this function. The chain length in cellular glycogen is precisely 13 glucose residues, as has been experimentally determined by several groups.

It is clear from eqns. (15) and (16) that the value of g_c that maximizes f is independent of the value of t (and consequently also of C_A), as t and g_c are independent variables (a glycogen molecule can have more or fewer tiers without modifying the chain length; this actually does occur during glycogen metabolism). On the other hand, as discussed below, there is a physical limit for t. Solving eqn. (15), we found that the optimum value of g_c that maximizes f from the root of $df/dg_c = 0$ is 12.93. This is well illustrated in Figure 6, in which the optimization function is plotted; note that the maximum of the function is $g_c \approx 13$;

therefore this is the chain length that optimizes the structure of glycogen.

Empirical data obtained by several groups are in good agreement with this theoretical result: Cori's group (Illingworth et al., 1952) reported data on 12 glycogens analysed from different sources, which gave an average chain length of between 10.8 and 15.4 glucose residues, with a mean value of 12.91 (for example: it was 13 in fetal guinea-pig liver and Sprague-Dawley rat liver and 12.5 in Busch strain rat liver and cat liver). Manners (1957) has noted that, of 84 different glycogens examined, 62 had chain lengths of 11-13 residues. Manners and Wright (1962) reported a chain length of 13 glucose residues in rabbit muscle and rat liver. Gunja-Smith et al. (1971) have reported chain lengths of 11.5 in human muscle, 12 in skate liver, 12.5 in Ascaris, 13 in rabbit muscle, 14 in cat liver and rabbit liver, 14.5 in Trichomonas foetus and 15 in horse diaphragm. Bullivant et al. (1983) have reported a value of 12-14 for white rabbit liver. An overall chain length of 12-14 glucose residues was given by Ryman and Whelan (1971) for the majority of glycogens [see also Smith (1968)]. By examining the optimization function in Figure 6, it is clear that these small deviations from 13 are not significant, and we can conclude that the chain length is an optimized parameter in glycogen structure.

DISCUSSION

The number of tiers in the glycogen molecule

In accordance with the data discussed here, Goldsmith et al. (1982) have calculated that the volume of the hypothetical 13th tier in the glycogen molecule would be 10000 nm³, and there would be about 55000 glucose residues in such a tier. Assuming an approximate volume of 0.113 nm³ for the glucose molecule (van der Waals volume), this would give 6215 nm³, which means that in that tier 62 % of the space would be occupied by glucose, leaving practically no space for phosphorylase. The same reasoning can be applied to glycogen-synthesizing enzymes, which would explain a very efficient way of controlling molecule size. Madsen and Cori (1958) calculated the surface area of the glycogen molecule according to the different number of tiers; they noted that the glucose residues become more and more crowded as the structure grows in size, and suggested that a structure of this type could be self-limiting in size. This conclusion is obvious. The existence of a physical limit to the size of the glycogen molecule is clear. The tier value of t = 12 is not just empirically known. The size of the enzymes involved in glycogen metabolism can explain it. We note with interest that in glycogenstorage disease type II (Pompe's disease), in which lysosomal amylo- $(1 \rightarrow 4)$ -glucosidase is lacking, there is a significant increase in glycogen in liver and muscle cells (see Stanbury et al., 1983). However, this is because of a large increase in glycogen particles accumulated in lysosomal vacuoles, not because of larger molecules (Baudhuim et al., 1964; Garancis, 1968).

Glycogen design and natural selection

We know that natural selection is one of the optimization mechanisms (maybe the only one) that works. The efficiency of this mechanism was analytically proven by Eigen and Schuster (1979) [see also Küppers (1983) and Morán and Montero (1984)]. During evolution, living organisms have to optimize functions with many variables, which have no explicit solution. Natural selection is really the application of optimization algorithms. The values of the glycogen molecule parameters, summarized in

Table 2, demonstrate that such a procedure was applied in the search for the optimum design of the glycogen molecule, and that this aim was successfully achieved.

Optimization principles have been used to study several aspects of cellular organization [see Heinrich et al. (1991) for a review]. The results reported here represent a new and unique case, i.e. the optimization of a molecular structure. A major point of interest of these results lies not only in their demonstration of the efficient design of glycogen but also in their revelation of the principal function of cellular glycogen. It is clear that glycogen is optimally designed to maximize the action of phosphorylase in anaerobic glycolysis; this in good agreement with the regulatory paraphernalia of this enzyme and with the specific design of anaerobic glycolysis [see distribution of lactate dehydrogenase isoenzymes in Kaplan (1964)].

These results lead us to another question: how did the cell evolve a glycogen molecule with these optimized features? It is clear that the specificity of the enzymes that synthesize glycogen determines the value of certain parameters, such as the minimum distance between branches; however, more than enzyme specificity is involved. The results reported by Smith (1968) suggest that the ratio between glycogen synthase and the branching enzyme is important in achieving the optimized design of glycogen: in a system of glycogen synthesis by chain elongation and branching, variation in the ratio of these enzymes leads to different degrees of branching. This is demonstrated by the glycogen-storage disease type IV (Andersen's disease) in which low activity of the branching enzyme produces a glycogen with very long outer branches. Furthermore, some 'abnormal' physiological conditions such as glucose or fructose infusion into rabbit liver produce glycogens with longer chains, e.g. $g_c = 16-17$ (Illingworth et al., 1952); rat liver glycogen shows similar differences depending on the amount of glycogen contained in the liver. These effects of external factors on glycogen structure suggest that some of the parameters of glycogen structure could be determined by the relative activities of the enzymes that synthesize it.

Starch appears to be designed to fulfil different functions. In general, our reasoning can only be applied to animal glycogen, in which the rapid response is of vital importance. The mechanisms that account for this rapid metabolism have not been observed in plants. Potato phosphorylase cannot be phosphorylated to give tetramers, is not stimulated by AMP, and in general lacks the regulatory and allosteric properties characteristic of muscle phosphorylase (ap Rees, 1974). The design of amylopectin (longer

Table 2 Values of the parameters of an optimized glycogen molecule and the actual values for cellular glycogen

Parameter	Optimum value	Cellular value
r	2*	2† 13‡
$g_{\rm c} t$	13* 12§	13‡ 12∥

- Values derived in this work.
- † Gunja-Smith et al. (1970).
- ‡ Illingworth et al. (1952); Manners (1957); Manners and Wright (1962); Gunja-Smith et al. (1971); Bullivant et al. (1983); and others, see the text.
 - § Physical limit in building the glycogen molecule.
 - Madsen and Cori (1958); Goldsmith et al. (1982).

chains) is rather different from that of glycogen. For example, chain length is 25 in potato and 22 in maize (Manners and Wright, 1962). It is probable that chain length in amylopectin has also been optimized, but its function is different from that of animal glycogen.

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