Naturwissenschaften (2011) 98:719–729
DOI 10.1007/s00114-011-0832-x

REVIEW

Glycogen with short average chain length enhances bacterial durability

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Received: 21 March 2011 / Revised: 22 July 2011 / Accepted: 22 July 2011 / Published online: 2 August 2011

Abstract Glycogen is conventionally viewed as an energy reserve that can be rapidly mobilized for ATP production in higher organisms. However, several studies have noted that glycogen with short average chain length in some bacteria is degraded very slowly. In addition, slow utilization of glycogen is correlated with bacterial viability, that is, the slower the glycogen breakdown rate, the longer the bacterial survival time in the external environment under starvation conditions. We call that a durable energy storage mechanism (DESM). In this review, evidence from microbiology, biochemistry, and molecular biology will be assembled to support the hypothesis of glycogen as a durable energy storage compound. One method for testing the DESM hypothesis is proposed.

Keywords Glycogen · Average chain length · Hidden Markov Model · Glycosidic linkage · Durability

Communicated by: Sven Thatje

Electronic supplementary material The online version of this article (doi:10.1007/s00114-011-0832-x) contains supplementary material, which is available to authorized users.

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Introduction

Bacterial energy reserves

In nature, (partially) free-living pathogens often encounter different types of stresses, such as hyperosmolarity, oxidative, DNA damage, and so on. Their survival depends on their ability to sense and respond to these changing stresses from time to time (Boor 2006). However, these functions require a comparatively large amount of energy, which is called energy of maintenance (Wilkinson 1963). Thus, an important factor for bacterial durability is their energy reserves, especially when considering that bacteria need to adapt to the large changes after shedding from hosts to the external environments and during invasion into hosts from external environments. Exceptions include, but are not limited to, those species that rely on spore formation to extend their survival in unfavorable conditions, such as *Bacillus clausii* and *Clostridium botulinum*. As a result, these bacteria may not require long-term energy reserves (also see the Electronic Supplementary Material).

So far, five major reserves have been identified as energy storage compounds in bacteria: triacylglycerols (TAGs), wax esters (WEs), polyhydroxybutyrate (PHB), polyphosphates (PolyPs), and glycogen. Although neutral lipids, TAG and WE, have high-energy capacity, their accumulations as intracellular lipid bodies are only found in a limited number of prokaryotes (Waltermann and Steinbuchel 2005; Kalscheuer 2010). PolyP and PHB are widespread polymers in bacteria. However, the linear structure of PHB makes it readily attacked by enzymes while PolyP can function as an ATP substitute (Zevenhuizen and Ebbink 1974; Kornberg et al. 1999). Thus, PolyP and PHB

primarily act as short-term energy suppliers. However, these comparisons are relative and exceptions do exist, which we will talk about later. Unlike the other four molecules, glycogen is a widespread and large-molecular mass (10^7–10^8 Da) polymer in bacteria (Wilson et al. 2010). Glycogen also has a highly and randomly branched structure, which makes it a more flexible energy reserve than other storage compounds. This will be explored below. Currently, glycogen has been reported to exist in more than 50 different bacterial species across all bacterial classes (Preiss 2009). For this review, we constructed a set of glycogen Hidden Markov Models (HMMs) (Eddy 1998) based on the general glycogen metabolism genes (*glgC*, *glgA*, *glgP*, *glgB*, *glgX*) from *Escherichia coli K12*. Using these, we screened 1,202 high-quality annotated bacterial proteomes from high-quality automated and manual annotation of microbial proteomes (HAMAP http://expasy.org/sprot/hamap/bacteria.html) (Lima et al. 2009) for glycogen metabolism genes in order to see the theoretical distribution of glycogen in bacteria (p-value cutoff 10^{-16}). In order to make sure our results are as accurate as possible, we also used protein annotations as references for HMM searching. We observed that 402 strains (245 species) have complete general genes for glycogen metabolism, while another 367 strains (234 species) do not have any of the genes (Electronic Supplementary Material).

Glycogen metabolism, bacterial lifestyle, and proteome sizes

Henrissat et al. screened 55 completely sequenced bacterial genomes and found that most of the free-living bacteria accumulate glycogen while those with parasitic lifestyles do not and concluded that glycogen accumulation could be a marker for bacterial parasitic behavior (Henrissat et al. 2002). Our study of the 1,202 bacteria in terms of existence or loss of glycogen metabolism pathways and bacterial lifestyle completes and extends the work of Henrissat et al. done on a more limited number of bacterial genomes. For example, most endosymbionts, such as *Buchnera aphidicola* and *Wolbachia pipientis*, and intracellular obligate pathogens, like *Coxiella burneti* and *Orientia tsutsugamushi*, have lost their glycogen synthesis and degradation abilities (see Electronic Supplementary Material for the complete list of bacterial lifestyles and distribution of glycogen metabolism pathway).

Genome reduction is correlated with bacterial lifestyle and habitat, which means that obligate intracellular pathogens are much more likely to have smaller genomes compared with free-living bacteria because potentially dispensable genes tend to be lost under selective pressure (Moran 2002; Sakharkar and Chow 2005). Based on these two theories, we compared the proteome sizes of bacteria with (402 proteome sizes) and without (367 proteome sizes) glycogen metabolism genes and found that average proteome size of the former group is significantly larger than that of the latter group (Fig. 1). Histograms of the two sets of bacteria give a detailed illustration of the distribution of proteome sizes (Fig. 2). Both suggest that bacteria with glycogen storage may have more varied lifestyles and occupy more diverse habitats and thus, on average, will be more durable. However, we must emphasize that there are exceptions. For example, *Acinetobacter* sp. and *Burkholderia pseudomallei*, which do not store glycogen at all but are able to utilize other energy storage compounds, are also rather durable in the external environment (Ishige et al. 2003; Inglis and Sagripanti 2006).

Durable energy storage mechanisms

Besides the existence or loss of glycogen metabolism pathways, glycogen branching structures vary. Certain types of glycogen are difficult to utilize and bacteria accumulating such glycogen are able to survive longer under starvation conditions when living freely in the external environment, while other types of glycogen are more easily broken down and the corresponding bacteria die at a higher

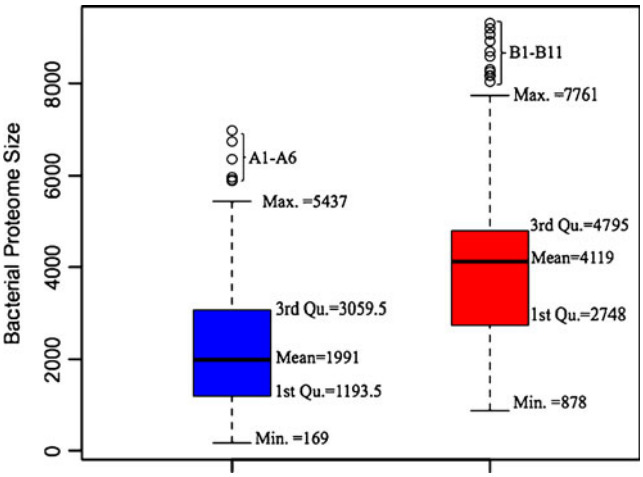

Fig. 1 Comparison of bacterial proteome sizes (sequences/proteome) between bacteria with (*red*) and without (*blue*) generally known glycogen metabolism genes. Proteome sizes are extracted from HAMAP bacterial proteome database http://expasy.org/sprot/hamap/bacteria.html. Difference between two sample groups (*blue* 367 proteomes; *red* 402 proteomes) is statistically significant (Welch two-sample *t* test value: t=16.5177, df=744.752, p-value <2.2e–16). *A1 Burkholderia cepacia* (6993), *A2 Hahella chejuensis* (6752), *A3 Ralstonia metallidurans* (6364), *A4 Delftia acidovorans* (5968), *A5 Conexibacter woesei* (5912), *A6 Brevibacillus brevis* (5887). *B1 Sorangium cellulosum* (9320), *B2 Amycolatopsis mediterranei* (9204), *B3 Rhodococcus sp.* (9075), *B4 Streptosporangium roseum* (8926), *B5 Streptomyces scabies* (8700), *B6 Burkholderia xenovorans* (8591), *B7 Stigmatella aurantiaca* (8307), *B8 Bradyrhizobium japonicum* (8253), *B9 Acaryochloris marina* (8172), *B10 Rhodococcus opacus* (8170), *B11 S. coelicolor* (8083)

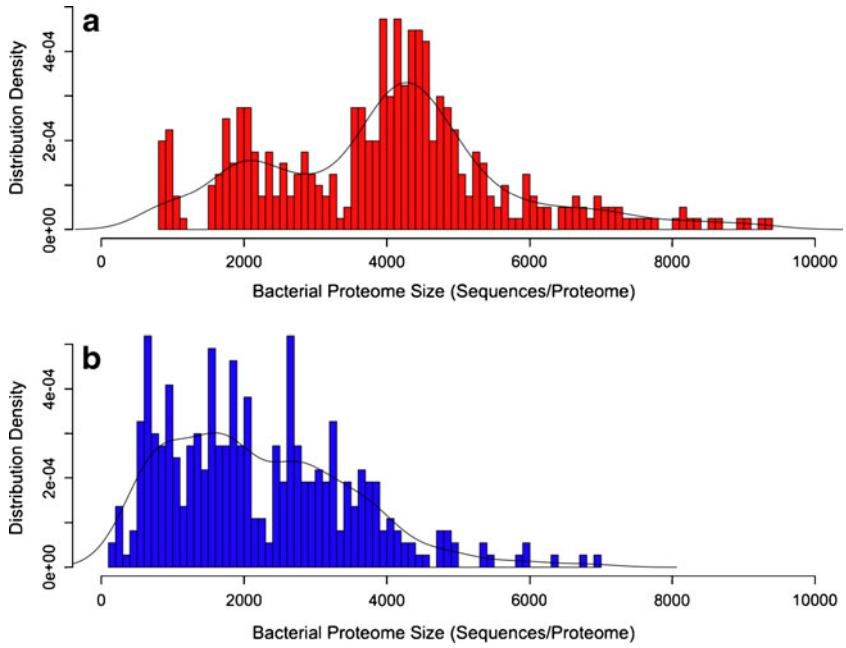

Fig. 2 Comparison of proteome size (sequences/proteome) distribution of bacteria **a** with or **b** without the complete set of glycogen metabolism enzymes by using histogram and distribution density: **a** 402 bacterial proteomes have complete glycogen metabolism enzymes while **b** 367 bacterial proteomes do not have any glycogen metabolism enzymes. From the figure, we could see the skewed distribution of bacterial proteome sizes in the two bacterial sets (Welch two-sample *t* test value: $t=16.5177$, $df=744.752$, p-value <2.2e–16)

rate when starved (Kim and Gadd 2008; Takata et al. 1998; Strange et al. 1961; Zevenhuizen and Ebbink 1974; Boylen and Mulks 1978; Strange 1968). Based on this phenomenon, we propose a hypothesis called the durable energy storage mechanism (DESM), by which we mean a storage compound forces bacteria to utilize the compound more slowly, e.g. due to steric inhibition, and thus enhances bacterial survival ability.

Studies of glycogen conformation versus degradation rate have shed some light on the relationship (Zevenhuizen and Ebbink 1974; Boylen and Mulks 1978; Takata et al. 1998), although there is as yet no direct experimental evidence. However, this inferred relationship gives us a starting point to look for more persuasive evidence for the existence of DESMs. In this review, we will look deeper into glycogen structure and bring together evidence from different fields to construct a framework for our DESM hypothesis. It is worth mentioning that glycogen is not the only candidate for the DESM hypothesis. WE is able to store more energy than TAG on a weight basis and is more resistant to degradation and oxidation, which could prolong bacterial survival during starvation and/or desiccation conditions (Finkelstein et al. 2010; Gurr et al. 2002). For example, some strains of *Acinetobacter*, e.g., *Acinetobacter baumannii*—a durable nosocomial pathogen—accumulate WE but not glycogen as an energy reserve (Ishige et al. 2003). Although the relationship between bacterial survival ability and WE still requires further exploration, it is reasonable for us to consider it as a possible candidate for our DESM hypothesis due to its physiochemical characteristics. However, in this review, we will focus on the more widespread bacterial molecule, glycogen.

Glycogen structure and its durability

Glycogen has been widely identified in and isolated from animals, fungi, and bacteria (Wilson et al. 2010). It is a water-soluble polymer consisting of α-D-glucosyl units. These units are connected together by α-1,4-glycosidic linkages to form oligosaccharide chains, with α-1,6-glycosidic linkages at branching points (Manners 1991). Unlike branches in amylopectin, which are discontinuously dispersed so amorphous and crystalline lamellae alternate, branches in glycogen are randomly and extensively distributed; no crystalline portion has ever been detected (D'Hulst and Merida 2010; Ball and Morell 2003), although some theoretical models assume that branched chains in glycogen are regularly distributed (Meléndez-Hevia et al. 1993). Using scanning tunneling microscopy Yang et al. (1990) reported that the glycogen molecule has a laminated structure.

Since glycogen was first extracted from liver tissue by Claude Bernard in 1857, its structure and function have been extensively studied (Young 1957). Although many models have been constructed, there is still no accurate description for glycogen structure due to its high molecular weight and random branches (Gunja-Smith et al. 1970; Manners 1991, 1957; Sullivan et al. 2010; Melendez et al. 1999). However, its basic framework has long been solved, and many parameters have been devised to describe glycogen structure, which are exterior chain (EC), interior chain, degree of polymerization, average chain length (ACL), etc. For details, please see the review paper by Manners (1991). Of all these parameters, glycogen ACL is one of the core factors impacting glycogen degradation rate

because it influences the percentage of α-1,6-glycosidic linkages and therefore the interaction with glycogen degrading enzymes (Park and Rollings 1995, 1994). Thus, ACL determines the durable characteristics of glycogen.

$$\mathrm{ACL} = \frac{\text{Number of Glucosyl Residues}}{\text{Number of Branching Points}}$$

Besides its function in balancing glucose homeostasis of blood, in animals, glycogen functions as a rapid and transient energy supplier for muscle activity (Kollberg et al. 2007; Melendez et al. 1999). Considering the consistency of its structural characteristics (Table 1) and the similarity of metabolic regulation, it is reasonable to conclude that glycogen is a transient energy source in animals. However, this conclusion may be not appropriate for bacterial glycogen due to the simpler structures and low energy requirements of bacteria, the wide variation of glycogen ACL (Table 1), and relatively low-level control mechanism of glycogen metabolism when compared with hormone control in higher organisms (Wilson et al. 2010; Oz et al. 2007; Tsintzas and Williams 1998).

Experimental data from different sources, summarized in Table 2, reveal a suggestive relationship between glycogen ACL and bacterial survival ability. For example, after examining glycogen metabolism in *Aerobacter aerogenes* (ACL=13) and *Escherichia coli* (ACL = 12), Strange (1968) concluded that "a feature of glycogen reserves in bacteria is their rapid depletion during starvation which suggests that any contribution glycogen makes towards maintenance and survival is of short duration". In contrast, glycogen in *Arthrobacter* (ACL = 7) and *Mycobacterium* (ACL = 7) is broken down very slowly (Zevenhuizen and Ebbink 1974). Coincidentally, both of the latter survive for a long time under starvation conditions (Zevenhuizen and Ebbink 1974; Boylen and Mulks 1978). Kim and Gadd (2008) also reports that *Arthrobacter globiformis* (ALC = 6.6) utilizes glycogen slowly and stays viable for a long time under starvation conditions while *E. coli* (ACL = 12) uses glycogen at a high rate and does not survive very long. In addition, Takata et al. (1998) also noted the faster degradation of glycogen in *Bacillus stearothermophilus* (ACL = 21) when compared with *E. coli* (ACL = 12).

Structural analysis of the bacterial glycogens above reveals a relationship between glycogen ACL and bacterial durability. It seems that short ACL (SACL) glycogen enhances bacterial durability under starvation conditions. Thus, what is it about SACL glycogen that makes it more difficult to break down? ACL and percentage of glycosidic linkages are interconnected. That is, for a glycogen with the same molecular weight, shorter ACL will lead to higher percentage of 1,6-glycosidic linkages, and vice versa. In this part, we discuss the influence of 1,6-glycosidic linkages on glycogen durability. The effects of glycogen ACL on glycogen durability will be explored in the next part, when talking about the substrate-enzyme interactions.

Takahash and Ono (1966) studied the heat of hydrolysis (ΔH) of α-1,4- and α-1,6-glucosidic linkages by calorim-

Table 1 Glycogen ACL in bacteria and eukaryotes

	Average chain length
Bacteria	
Aerobacter aerogenes	13
Agrobacterium tumefaciens	13
Arthrobacter globiformis	6.6
Arthrobacter spp.	7~9
Bacillus megaterium	10~11
Bacillus stearothermophilus	21
Chromatium strain D	11.1~11.4
Clostridium botulinum	17
Desulfurococcus	7
Escherichia coli	12, 10.8, 13, 14
Klebsiella pneumoniae	11.6
Mycobacterium tuberculosis	7~9, 10~12
Neisseria perflava	11~12
Nostoc muscorum	13
Prevotella ruminicola	8
Pseudomonas V-19	8
Selenomonas ruminantium	12, 23.5
Sphaerotilus natans	13
Streptococcus mitis	12
Sulfolobus	7
Synechocystis sp. *PCC6803*	7.5~10.4
Thermococcus	7
Thermoproteus	7
Thermus thermophilus	7
Eukaryotes	
Bass liver	14
Bullhead liver	12
Cat liver	13
Dog liver	12
Haddock liver	12
Horse liver	11
Horse muscle	11~12
Human muscle	12
Northern pike liver	12~13
Rabbit muscle	11~13

Data obtained from: Abdelakher and Smith (1951), Bender (1979), Boeck and Schinzel (1998), Builder and Walker (1970), Chao and Bowen (1971), Hara et al. (1973), Kamio et al. (1981), Kent and Stacey (1949), Konig et al. (1982), Lou et al. (1997), Manners (1957), Scherp (1955), Takata et al. (1998), Wallace (1980), Weber and Wober (1975), Whyte and Strasdin (1972), Yoo et al. (2007), Zevenhuizen (1992), Zevenhuizen and Ebbink (1974)

Table 2 The relationship between glycogen ACL and bacterial survival time

Bacterial name	Average chain length	50% Survival time
Aerobacter aerogenes	13	45h
Arthrobacter globiformis	6.6[a]	20d[a]
Arthrobacter spp.	7~9	80d
Bacillus megaterium	10	20d[ab]
Escherichia coli	12	36h
Klebsiella pneumoniae	11.6[a]	2.5d[ab]
Mycobacterium tuberculosis	7~9	52d[a]
Pseudomonas V-19	8	60d
Streptococcus mitis	12[a]	22h
Thermococcus	7[a]	24.5d[ab]

The table is adapted from Zevenhuizen and Ebbink (1974). Additional data obtained from: Bender (1979), Boylen and Mulks (1978), Builder and Walker (1970), Lappinscott et al. (1988), Lopez et al. (1998), Konig et al. (1982), Takahata et al. (2001),Walther and Ewald (2004), Zevenhuizen (1992)

[a] Additional data from cited references

[b] Data produced by averaging over points in graphs from cited references

etry and found that $\Delta H_{1,4}$ was −1,100 cal/mol and $\Delta H_{1,6}$ was +1,300 cal/mol at 25°C, which was further confirmed by Tewari and Goldberg (1989). Although these results were obtained by studying maltose (disaccharides linked with α-1,4-glycosidic bonds) and isomaltose (disaccharides linked by α-1,6-glycosidic bonds), the heat of hydrolysis of polysaccharides is an additive function of the thermal data of the individual linkages (Takahash and Ono 1966). Thus, it is reasonable to apply these thermal data to glycogen analysis. Glycogen branching enzyme (EC = 2.4.1.18) (GBE), the principal enzyme responsible for the formation of branched side chains has two functions: breaking the α-1,4-glycosidic linkages from one chain and transferring oligosaccharides to the same or other chains by forming α-1,6-glycosidic linkages (Abad et al. 2002). In this process, one α-1,4-linkage is broken and one α-1,6-linkage is generated. Thus, the whole process will release −2,400 cal/mol energy, and the branching reaction is thermodynamically favored. As French (1964) concluded, "in an enzymically catalyzed system, if there was a free opportunity to exchange α-1,4- to α-1,6-bonds reversibly, the equilibrium mixture would probably contain 70–90% of α-1,6-bonds." The reason why this does not happen in glycogen is probably due to its spatial self-limitation, that is, a large number of α-1,6-glycosidic bonds leads to more branches and so higher density, which restricts further branching of glycogen (Meléndez-Hevia et al. 1993). However, a specific explanation still requires further research.

On the other hand, ΔH can also be used as an indicator for determining the relative stabilities of isomeric compounds (Gallagher et al. 1998). For example, branched hydrocarbon isomers release more heat when forming than less branched and straight chains. Experiments and theoretical calculation have shown that branched hydrocarbon isomers are more energetically stable than straight chains and that isomer stability increases with branching of the hydrocarbon (Laidig 1991). Thus, from the standpoint of glycogen synthesis, we infer that highly branched glycogen is more stable than less branched glycogen and therefore more durable when undergoing degradation because more alpha-1,6-glycosidic linkages exist in hyperbranched glycogen. Experiments are required to confirm this conclusion.

Acid hydrolysis of glycogen excludes the influences of substrate–enzyme interactions and has revealed the inherent characteristics of glycogen durability. It was found that the non-reducing end α-1,4-linkages are hydrolyzed at a faster rate than the other α-1,4-linkages, and α-1,4-linkages are hydrolyzed four times faster than α-1,6-linkages at 100°C, and seven times faster at room temperature (Wolfrom et al. 1951; Erlander and French 1958). In order to confirm that linkage differences would be sufficient to change degradation rates, one need only look at the hydrolysis of various polysaccharides in acid solution (7.7 N HCl at 30°C), including amylose, amylopectin, glycogen, limit dextrin (a more highly branched polysaccharide than glycogen), and dextran (polysaccharide with α-1,6-glycosidic linkages only). Results showed a significant correlation between percentage of α-1,6-linkages in polysaccharide and resistance to acid hydrolysis (Swanson and Cori 1948). In sum, glycogen with higher percentage of α-1,6-glycosidic linkages should be more thermodynamically stable and harder to break down.

Glycogen metabolism and enzymology

Enzymatic reaction kinetics is known to be affected by substrate structures (Park et al. 1988). Since glycogen is a polydisperse polymer, we would expect that its conformation, which is determined by its average chain length (Table 1), has a significant influence on the substrate-enzyme interactions and will further influence its degradation rate. Five enzymes are generally important in the process of glycogen metabolism, although some of them are not essential in some bacteria: ADP-Glucose pyrophosphorylase (EC = 2.7.7.27), glycogen synthase (EC = 2.4.1.21), α-1,4-glucan-branching enzyme (EC = 2.4.1.18), glycogen phosphorylase (EC = 2.4.1.1) and glycogen debranching enzyme (EC = 3.2.1.-) (Ballicora et al. 2003; Ball and Morell 2003; Buschiazzo et al. 2004; Abad et al. 2002; Alonso-Casajus

et al. 2006; Dauvillee et al. 2005). Figure 3 shows a schematic model for glycogen metabolism. Although each enzyme has its unique function, study of the corresponding structural genes shows that these enzymes work together to maintain a balance between glycogen biosynthesis and degradation, which leads to the different glycogen conformations in bacteria (Ballicora et al. 2003; Ball and Morell 2003; Buschiazzo et al. 2004; Abad et al. 2002; Alonso-Casajus et al. 2006; Dauvillee et al. 2005). In addition, variation of glycogen structure is also reported to correlate with development stages and growth conditions of microorganisms (Yoo et al. 2007; Norrman et al. 1975). By reviewing the interactions of α-amylase (EC = 3.2.1.1), glycogen phosphorylase, and glycogen debranching enzyme with various polysaccharides, we see how substrate structures are related to degradation rates.

α-Amylase is an enzyme that hydrolyses α-1,4-glycosidic linkages in oligosaccharides and polysaccharides (Park and Rollings 1994). It mainly exists in animals and plants and is not involved in bacterial interior glycogen breakdown. In fact, many bacteria secrete α-amylase into the environment to digest exogenous amylopectin and amylose (Shelburne et al. 2009; Raha et al. 1992) and isoamylase to digest glycogen, amylopectin, and their beta-limit dextrins (Amemura et al. 1988), while pullulanase is secreted to the outer membrane to break down pullulan, amylopectin, and glycogen (Kornacker and Pugsley 1990). In addition, both *E. coli* α-amylase over-expressors and mutants impaired in α-amylase have normal glycogen content (Eydallin et al. 2007b). However, α-amylase is a good example to explain how substrate structure can influence its degradation rate when interacting with enzymes. Unlike β-amylase (EC = 3.2.1.1) and glycogen phosphorylase that only work on the non-reducing ends of glycogen, α-amylase catalyzes the breakdown of α-1,4-glycosidic bonds inside polysaccharides and forms α-amylase resistant macro-dextrin and oligosaccharides (Brammer et al. 1972). In order to understand the effects of branching substrate on α-amylase activity, it would be much clearer if we had experimental data on α-amylolysis of glycogens with different branching degrees. However, no such experiments have ever been done. Park et al. (1988) studied this problem by using three closely related polysaccharides, amylose, amylopectin, and glycogen, the main difference being their average chain lengths. In addition, they used aqueous size exclusion chromatography with low-angle laser light scattering to detect polysaccharide molecular weights and distribution of branching characteristics. Reaction rates of polysaccharide amylolysis decreased with the increase of branching degree of polysaccharides, that is, decrease of average chain length (Park and Rollings 1994, 1995). Further kinetic modeling based on the data obtained above agreed well with experimental data, which implied a significant relationship between enzyme action and structural properties of polymeric substrate (Park and Rollings 1994, 1995).

Average chain length of glycogen also has a significant effect on the activities of glycogen phosphorylase and debranching enzyme. It has generally been accepted that glycogen phosphorylase in bacteria degrades glycogen from non-reducing ends of the outer layer down to a length of four glucosyl residues away from the first branching points encountered by the enzyme (Alonso-Casajus et al. 2006). Branched chains less than or equal to four glucosyl residues are highly resistant to phosphorylase and can only be degraded by a glycogen debranching enzyme at a comparatively lower rate (Dauvillee et al. 2005; Kim and Gadd 2008). In addition, branched chains with more than four glucosyl units cannot be catalyzed by glycogen debranching enzyme, which avoids generating an extensive futile cycle during glycogen synthesis, because branching enzyme transfers oligosaccharides with 5 to 11 glucosyl residues (Dauvillee et al. 2005). For example, Takata et al. (1998) shows that glycogen phosphorylase has a high affinity to glycogen if the average chain length is longer. Recall that the ACL of glycogen in *B. stearothermophilus* is 21 but in *E. coli* it is only 11–14. Experiments revealed that the affinity of glycogen phosphorylase for *E. coli* glycogen is comparatively lower while glycogen phosphorylase in *B. stearothermophilus* has about 100-fold higher affinity to glycogen and higher specific activity (Takata et al. 1998). Similarly, the activity of glycogen phosphorylase in *E. coli* is relatively higher than that in *Arthrobacter* spp. (ACL 7–9) (Kim and Gadd 2008). In sum, SACL glycogen is harder to degrade than glycogen with normal or longer ACL.

Glycogen metabolism-related genes and glycogen structure

With the development of molecular biology, more and more effort has been put into the study of structural genes of glycogen metabolism and their influence on glycogen structure (Buschiazzo et al. 2004; Dauvillee et al. 2005; Alonso-Casajus et al. 2006; Ballicora et al. 2003; Binderup et al. 2000). The corresponding genes for enzymes of glycogen metabolism are *glgC*, *glgA*, *glgB*, *glgP*, *glgX*, respectively (Fig. 3). In addition, although the organization of the genes involved in glycogen metabolism differs across bacterial species, the genes are generally organized in one or more operons (Cho et al. 2008). For example, they are organized in a single operon in *E. coli*: *glgBXCAP* and, within *glgC*, an alternative suboperonic promoter further directs *glgAP* expression (Montero et al. 2010). Two other related genes, *glgD* and *glgS*, are also found in bacteria. However, they are not essential for most glycogen-

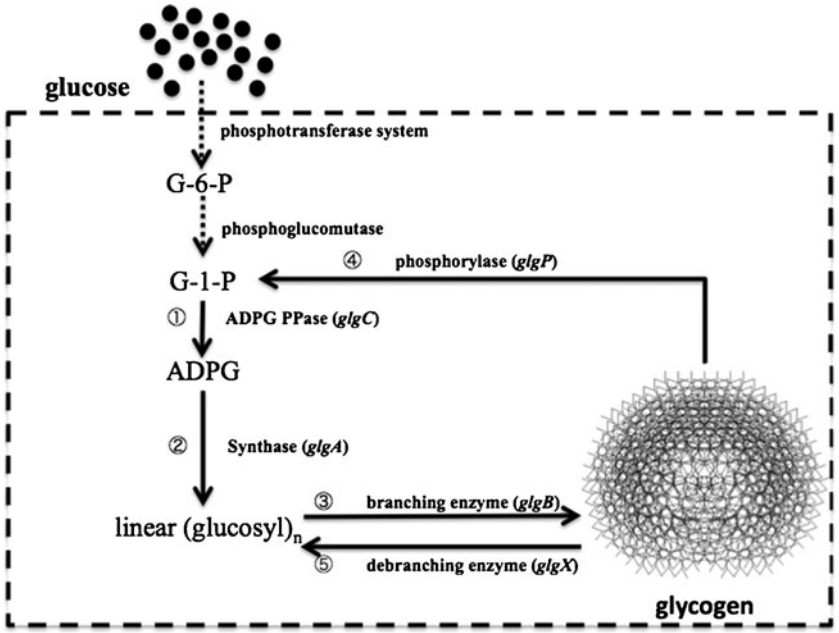

Fig. 3 Schematic illustration of glycogen metabolism. The numbered enzymes are generally important for glycogen synthesis and degradation. Corresponding gene names are in parentheses. The onion-shaped glycogen model is drawn based on the simplified fractal model of glycogen structure by using Python script and only for illustrative purpose

producing bacteria. *glgD* is a gene for the biosynthesis of a subunit of a heterotetrameric ADP-Glucose pyrophosphorylase, while *glgS* is able to stimulate glycogen biosynthesis when overexpressed (Kozlov et al. 2004; Takata et al. 1997).

Recent research has confirmed that glycogen metabolism is a sophisticated network and is highly interconnected with many cellular processes (Eydallin et al. 2007b; Wilson et al. 2010). In this part, we will not look into the network as a whole because it is not related to our topic. Instead, we focus on the five important genes and review their effects on glycogen structure when intentionally mutated. Manipulation of glycogen-related genes provides us with a direct and convenient method to test the glycogen-related DESM hypothesis (other DESMs are still under investigation).

GlgP and GlgX are essential for the degradation of glycogen. Deletion would lead to the over-accumulation of glycogen (Eydallin et al. 2007b). Bacterial strains with mutations in these genes are unable to utilize glycogen properly, and both of the gene products do influence the structure of glycogen. *glgP* mutants of *E. coli* accumulate glycogen with longer average chain length while *glgX* mutants have shorter chains in the outermost layer of glycogen (Alonso-Casajus et al. 2006; Dauvillee et al. 2005). On the other hand, mutation of *glgA* would lead to the absence of glycogen because GlgA is responsible for the elongation of linear chains, so without it, there would be no glycogen (Eydallin et al. 2007b). It is worth mentioning that, although *glgC*, encoding ADP-glucose (ADPG) pyrophosphorylase (E.C.2.7.7.9) that converts glucose-1-phosphate to ADPG, is an important source for ADPG accumulation, it is not an essential one. Recent studies have shown that enterobacteria have more than one source of ADPG for glycogen biosynthesis, but the additional sources have not yet been identified (Moran-Zorzano et al. 2007; Eydallin et al. 2007a). Martin et al. also pointed out that a GlgC is only essential for the first of the two phases of glycogen synthesis in *Streptomyces coelicolor* A3(2) (Martin et al. 1997).

Thus, to address average chain length, only *glgB*, encoding glycogen branching enzyme, is suitable for genetic manipulation because *glgB* mutation will lead to the variation of average chain length of glycogen without impacting the synthesis and utilization pathways (Lares et al. 1974; Binderup et al. 2000, 2002; Guan et al. 1995; Devillers et al. 2003). It has also been observed that branching enzymes from different sources show considerable variation of specific activities, chain transfer patterns, and substrate preferences of branching enzymes (Binderup et al. 2000). Bacteria without *glgB* tend to produce amylose-like polysaccharide instead of hyper-branched glycogen, and the amount of glycogen produced is much less (Lares et al. 1974). In addition, recent studies have revealed that progressively reducing the length of N-terminal domain of glycogen branching enzyme of *E. coli* results in the variation of chain transfer pattern, which further impacts the distribution of chain lengths in glycogen, that is, glycogen ACL (Binderup et al. 2000, 2002; Devillers et al. 2003). Finally, the type of N-terminal domain (unmodified) will also have an impact on glycogen ACL (Palomo et al. 2009). However, all these results were obtained by in vitro experiments, where engineered glycogen branching enzymes (progressively N-terminal truncation or extraneous N-terminal) were expressed in bacteria and then extracted to

react with amylose in vitro (Devillers et al. 2003; Palomo et al. 2009). Based on these experimental data, one could manipulate the structure of glycogen by changing the length of GlgB in *E. coli* or construct hybrid GlgB by inserting short-chain preferred N-terminal domain, while other factors are kept constant. In this way, average chain length is the single variable but the total amount of glycogen is not changed much by the GlgB mutation. Then, one can study the viability of *E. coli* under starvation conditions and see the effects that glycogens with different ACL have on the survival time of *E. coli*.

Previous work done by Devillers et al. (2003) showed that branching enzymes with N-terminal 112 residues deleted (Nd_{1-112}) had lower catalytic efficiency than wild-type branching enzymes, while their substrate specificity was the same. A histogram shows the distribution of chain lengths for different N-terminal deletions (Fig. 4) (Devillers et al. 2003). Below are the formulas (formula a and formula b) calculating the weighted ACL for the wild-type and Nd_{1-112} deletion strains. Numbers used to calculate glycogen ACL are estimated based on the histogram (Fig. 4).

Formula a. Glycogen ACL of WT strain

$$\frac{7.5 \times 20 + 12 \times 43 + 17 \times 27 + 23 \times 7 + 28 \times 2 + 35.5 \times 1}{100} = 13.375 \approx 14$$

Formula b. Glycogen ACL of Nd_{1-112} strain

$$\frac{7.5 \times 5 + 12 \times 24 + 17 \times 30 + 23 \times 16 + 28 \times 12 + 35.5 \times 13}{100} = 20.01 \approx 20$$

Specifically, progressively reducing the length of GBE N terminus leads to the shift of distribution of the transferred chains toward longer lengths in vitro. Palomo et al. (2009) constructed chimeric GBEs from *Deinococcus geothermalis* and *Deinococcus radiodurans* to study the effects N-domain had on the structure of glycogen, results of which indicated that the N-terminus of this enzyme determines not only glycogen branching pattern but also substrate specificity. The latter conclusion is inconsistent with the result obtained from *E. coli* experiments mentioned above (Binderup et al. 2000).

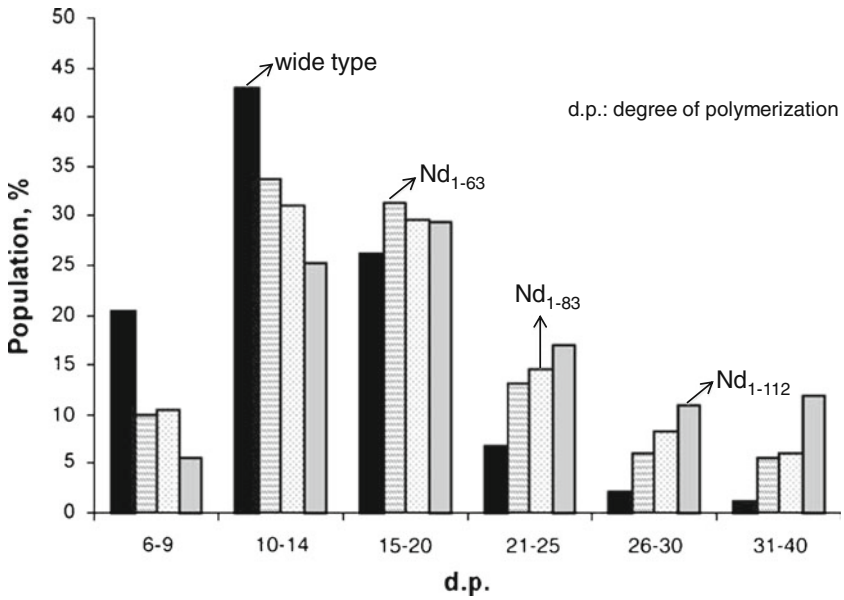

Fig. 4 Shift of distribution of chain lengths of glycogen after 2 h of reaction with isoamylase in vitro (from Devillers et al. (2003)). Amylose was treated by GBEs with different chain transfer patterns. Then, isoamylase was used to degrade glycogen at α-1,6-glycosidic linkages. Finally, distribution of chain lengths was checked by HPAEC. The histogram shows the changes of the distribution of chain lengths of glycogen. Based on this data, calculation of average chain length reveals an apparent change of ACL between WT strain (ACL ≈ 14) and Nd1–112 mutant (ACL ≈ 20). Please see body text for formulas

Conclusions and prospects

Glycogen is a highly branched homopolysaccharide that is widely distributed across bacterial species as an energy and carbon source. Moreover, loss of glycogen metabolism has been considered as a common marker for parasitic behavior, and most of these parasitic bacteria are either obligate intracellular pathogens or symbionts within hosts. This review reveals that bacterial glycogen with different ACL degrades at different rates. That is, the shorter the average chain length, the slower the breakdown rate, which in turn enhances bacterial durability. We call this enhancement a DESM. Finally, a feasible method is proposed to test glycogen as a durable energy storage compound.

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