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Thermodynamic Function of Glycogen in Brain and Muscle

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Abstract:

Key features of glycogen metabolism in excitable tissues are not well-explained by current concepts. Glycogen stores in brain and skeletal muscle are generally considered to function as local glucose reserves, to be utilized during transient mismatches between glucose supply and demand; however, quantitative measures show that blood glucose supply is likely never rate-limiting for energy metabolism in either brain or muscle under physiological conditions. These tissues nevertheless do normally utilize glycogen during intervals of increased energy demand, despite the availability of free glucose, and despite the ATP cost of cycling glucose through glycogen polymer. This seemingly wasteful shunt can be explained by considering the effect of glycogenolysis on the amount of energy derived from ATP (ΔG'ATP). ΔG'ATP is diminished by elevations in Pi, such as occur at sites of rapid ATP hydrolysis and net phosphocreatine consumption. Glycogen utilization counters this effect by sequestering Pi in glycolytic metabolites ($glycogen_n$ + Pi → $glycogen_{n-1}$ + glucose-1-phosphate → phosphorylated glycolytic intermediates), and thereby maintains the amount of energy obtained from ATP at sites of rapid ATP consumption. This thermodynamic effect may be particularly important in the narrow, spatially constricted astrocyte processes that ensheath neuronal synapses. This effect can also explain the co-localization of glycogen and cytosolic phosphocreatine in brain astrocytes, glycolytic super-compensation in brain when glycogen is not available, and aspects of exercise physiology in muscle glycogen phosphorylase deficiency (McArdle's disease).

Keywords: astrocyte; ATP; brain; exercise; glucose; glycogen; McArdle's disease; muscle, neuron; phosphocreatine; seizure

Introduction

Glycogen is a highly branched polymer of glucose (Figure 1). It is stored at high concentrations in liver as a systemic glucose reserve, but also present in skeletal muscle and brain. Glycogen in these tissues is considered to be a local energy reserve, serving to buffer transient mismatches between glucose supply and demand. However, this concept fails to account for key aspects of glycogen metabolism. In brain, glycogen metabolism occurs continuously, despite a net ATP cost relative to free glucose utilization. In skeletal muscle, glycogen is required for rapid and efficient contraction even when free glucose is available; and in both brain and muscle, local glucose concentrations rarely if ever fall to levels that could be rate-limiting for energy metabolism.

The prevailing view also ascribes differing and somewhat conflicting roles for glycogen in muscle and brain. In muscle, strenuous exercise induces a massive increase in ATP utilization that is met largely by an increased rate of glycolysis and lactate production. Glycogen can be used to fuel glycolysis, and depletion of muscle glycogen correlates with muscle fatigue. These observations underly the established view that muscle glycogen serves as an auxiliary, local store of glucose that is utilized to fuel rapid glycolytic metabolism. However, there is no evidence that glucose supply to exercising muscle is rate limiting for energy production; in fact, glucose levels in muscle increase as a result of augmented blood flow. This view is also difficult to apply to brain, where glycogen turnover is continuous and occurs primarily in astrocytes rather than neurons. Glycogen metabolism in astrocytes is regulated by neurotransmitters and other signaling molecules and is thus viewed as a source of lactate for activated neurons, rather than an energy substrate for the astrocytes themselves. However, recent quantitative studies indicate that most lactate derived from astrocyte glycogen is released to the blood stream, as is the lactate generated from exercising muscle. Also as in muscle, there is no evidence that brain glucose consumption in brain can outstrip normal supply. These interleaving considerations warrant a reassessment of the basic observations and assumptions pertaining to glycogen metabolism in both brain and muscle. Here we review the bioenergetics of glycogen metabolism, identify conflicts between current concepts and experimental data, and argue that the primary function of glycogen is the same in both brain and skeletal muscle: to maintain maximal energy yield from ATP.

The capacity of cells to carry out energy-dependent processes depends not only on their capacity to maintain ATP concentrations, but also on the amount of energy that can be obtained from ATP hydrolysis ($\Delta G'_{ATP}$). $\Delta G'_{ATP}$ is influenced by the ratios of the reactants and products of the ATP hydrolysis reaction; in simplified form, $\Delta G'_{ATP}$ varies in proportion to [ADP]·[Pi] / [ATP]. As this ratio rises the $\Delta G'_{ATP}$ becomes smaller, and at the extreme condition no energy can be obtained regardless of ATP abundance (Nicholls and Ferguson, 2013). Crucially, the first step of glycogen mobilization subtracts inorganic phosphate (Pi) from the cytosol (Figure 2), thereby preserving $\Delta G'_{ATP}$. This thermodynamic function of glycogenolysis is likely of particular importance for cells that episodically consume ATP at very rapid rates.

Control and bioenergetics of glycogen metabolism

Glycogen concentrations vary considerably across cell types (Table 1). They are highest in liver, brain and skeletal muscle, and much lower in smooth muscle and other tissues. Liver has a special role as a systemic glycogen storage depot. To this end, hepatocytes express high levels of glucose-6-phosphatase for generating exportable free glucose from glycogen-derived glucose-6-phosphate (Figure 1). Brain and muscle have only negligible expression of glucose-6-phosphatase (Dringen et al., 1993; Gamberucci et al., 1996), and do not have a role analogous to hepatocytes as a source of free glucose for use by other cells.

As diagrammed in Figure 2, the glucose residues in glycogen are joined by α-1,4-glycosidic bonds, with branch points formed by α-1,6-glycosidic bonds at approximately every 10 - 14 glucose residues (Calder, 1991). Glycogen synthesis requires uridine diphosphate glucose (UDP-glucose) as a substrate, which is produced from uridine triphosphate and glucose-1-phosphate by UDP-glucose pyrophosphorylase. The α-1,6-glycosidic branch points are subsequently produced by glycogen branching enzyme (1,4-alpha-glucan-branching enzyme). Glycogenolysis is mediated by glycogen phosphorylase, which hydrolyzes glucose residues at α-1, 4 linkage points to generate glucose-1-phosphate. Glycogen debranching enzyme acts at the α-1, 6 branch points to provide a linear substrate for glycogen phosphorylase (Nakayama et al., 2001).

There are three isoforms of glycogen phosphorylase in mammals: a liver isoform (PYGL), a muscle isoform (PYGM) and a brain isoform (PYGB), each termed by the tissue in which it is predominately expressed. In brain, PGYB and also PGYM and are abundantly expressed in astrocytes, with low levels of PGYB also detectable in some neurons (Pfeiffer-Guglielmi et al., 2003). The isoforms differ in response magnitude to various regulatory influences (Mathieu et al., 2017). Glycogen phosphorylase is regulated by changes in energy state through allosteric effects of AMP, which accelerates enzymatic activity, and ATP and glucose-6-phosphate, which slows activity. Glycogen phosphorylase is also regulated through phosphorylation by glycogen phosphorylase kinase, which is in turn responsive to several signaling pathways. For example, stimulation of ß-adrenergic receptors activates glycogen phosphorylase kinase through the cAMP / protein kinase A pathway. This provides a mechanism for “anticipatory” glycogen mobilization prior to any perturbation in cellular energy state. Glycogen phosphorylase kinase can also be activated by Ca^{2+} binding to its calmodulin subunit, a mechanism particularly important in skeletal muscle, where contraction is initiated by release of Ca^{2+} from the sarcoplasmic reticulum.

The immediate product of glycogen phosphorylase is glucose-1-phosphate, which exists in equilibrium with glucose-6-phosphate. Glucose-6-phosphate is also produced from free glucose in the initial step of glycolysis (Figure 1), but there are significant bioenergetic differences between these two pathways. First, there is a net cost of one ATP for each glucose residue shuttled onto and off glycogen polymer, as two ATP are consumed in forming UDP-glucose and only one ATP equivalent is regained with the formation of glucose-1-phosphate (Figure 2). Second, glycogen metabolism to glucose-1-phosphate removes Pi from the cytosol, whereas formation of glucose-6-phosphate from free glucose

does not. These two differences have fundamental implications for the potential functions of brain and muscle glycogen.

Glycogen metabolism in brain

The importance of glycogen in brain function is less widely recognized than its role in muscle. Astrocytes contain most glycogen in brain, with much smaller amounts also detectable in meningeal cells, endothelial cells, and other cell types (Cali et al., 2016; Cavalcante et al., 1996; Ibrahim, 1975; Koizumi, 1974). Neurons contain appreciable amounts of glycogen during development, but this falls to low levels in the mature brain except in certain brainstem neurons (Borke and Nau, 1984; Oe et al., 2016; Saez et al., 2014). Astrocytes form a syncytium with one another and actively maintain homeostasis of brain extracellular space (Kettenmann and Ransom, 2012). They extend innumerable thin processes around neuronal structures and function independently of one another as metabolic microdomains (Grosche et al., 1999). Electron microscopy identifies glycogen granules throughout astrocyte cell bodies and processes, particularly near axonal boutons and dendritic spines (Cali et al., 2016). After accounting for the fact that almost all brain glycogen is in astrocytes, and astrocytes comprise less than 25% of brain volume (Gundersen et al., 2015), the estimated glycogen concentration in astrocytes is roughly comparable to that in skeletal muscle (Table 1).

Glycogen in astrocytes can support neuronal function and survival and function for limited periods of time during energy failure caused by profound hypoglycemia or brain ischemia (Kilic et al., 2018; Seidel and Shuttleworth, 2011; Suh et al., 2007; Swanson and Choi, 1993; Swanson et al., 1990; Wender et al., 2000), but several factors indicate this to be not its primary function. These factors include continuous turnover of brain glycogen during normal brain activity, suppression of glycogen turnover during anesthesia or hibernation (Swanson, 1992), the location of most glycogen in astrocytes rather than neurons, and the complex neuromodulatory influences that regulate astrocyte glycogen metabolism. Brain glycogen turnover (utilization and re-synthesis) accelerates in response to local neuronal activity (Swanson et al., 1992; Watanabe and Passonneau, 1973) to rates that can match or exceed the local increase in glucose metabolism (Dienel, 2019; Shulman et al., 2001). The turnover is stimulated by neurotransmitters and other signaling molecules including serotonin, norepinephrine, arachidonic acid, glutamate, and K^+ (Magistretti, 1988; Quach et al., 1982; Subbarao et al., 1995; Walls et al., 2009).

Studies of transgenic mice lacking glycogen synthase in brain also demonstrate a physiological importance of brain glycogen. These mice are overtly normal, but have increased susceptibility to hippocampal seizures following the administration of kainate or stimulation of Schaffer collaterals (Lopez-Ramos et al., 2015). These mice also exhibit impaired synaptic plasticity, as evidenced by decreased long-term potentiation in the hippocampus and impairment in an associative learning task (Duran et al., 2013). Studies using inhibitors of glycogen phosphorylase likewise suggest a role for glycogen in memory consolidation (Gibbs et al., 2006; Gibbs and Hutchinson, 2012; Gibbs et al., 2007; Newman et al., 2011). Electrophysiology studies of *ex vivo* optic nerve and corpus callosum show that the capacity of axons to

maintain conductance during high frequency stimulation varies with the concentrations of astrocyte glycogen in these structures (Brown et al., 2012; Brown et al., 2005; Wender et al., 2000). These and other studies also suggest that lactate derived from astrocyte glycogen can ultimately be metabolized by neurons (Dringen et al., 1993; Tekkok et al., 2005; Tsacopoulos and Magistretti, 1996); however, lactate metabolism is ubiquitous (Veech, 1991), and there remains controversy as to whether this is a significant mechanism by which astrocyte glycogen influences neuronal function (Dienel, 2019; Dienel and Cruz, 2004).

Current concepts about glycogen metabolism in brain

The energetic cost of shuttling glucose on and off glycogen limits the conditions under which there can be an advantage to utilizing glycogen stores over free glucose. One such condition is during transient, local insufficiencies in glucose supply, as might occur in the brief interval between a burst of neuronal activity and subsequent increase in local blood flow. This idea was proposed by Swanson (Swanson, 1992), and more formally developed by Shulman and colleagues as the “glycogen shunt” hypothesis to explain the transient increase in the ratio of glucose : oxygen utilization observed during local brain activation (Shulman et al., 2001). A related idea proposes that astrocyte glycogenolysis occurs pre-emptively near active neurons to prevent reductions in neuronal access to extracellular glucose (Dienel and Cruz, 2015; DiNuzzo et al., 2010). Although these concepts have gained wide support, experimental observations suggest than glucose availability is rarely, if ever rate-liming for energy production. Brain glucose concentration is normally 1 - 2 mM (de Graaf et al., 2001; Gruetter et al., 1998; Shestov et al., 2011), and reductions in this concentration cannot significantly limit glucose metabolic rate unless they fall to values near the hexokinase Km for glucose, which is 0.03 - 0.3 mM (Wilson, 2003). The classic study by Silver and Ericińska found that spreading depression, which causes nearly simultaneous bursting of neurons at its wave front and thus approximates the maximal rate of brain energy demand, reduced extracellular glucose concentrations by only 25% (Silver and Erecinska, 1994). Pentylenetetrazole-induced status epilepticus similarly produces only minor reductions in brain glucose concentration (Folbergrova et al., 1985; Ingvar et al., 1984; McCandless et al., 1987), excluding settings in which cerebral blood flow is impaired. These observations are consistent with estimates that the maximum rate of glucose transport in brain is more than double the maximum rate of glucose utilization (de Graaf et al., 2001; Shestov et al., 2011).

These experimental findings cast doubt on the concept that normal brain activity can drive local glucose concentrations below levels required to fuel neuronal or astrocyte function even for very brief time intervals, and suggest instead that glycogen must serve a purpose other than (or in additional to) a local glucose reserve. This concept is supported by studies using cell culture preparations, in which glucose is at all times available in far excess of need. In cultured astrocytes, the maximal rate of energy-dependent K^+ uptake is reduced by pharmacological inhibition of glycogen phosphorylase (Xu et al., 2013). Glycogen phosphorylase inhibition similarly reduces miniature excitatory and inhibitory

postsynaptic currents in neurons cultured with astrocytes, but not in neuron cultures without astrocytes (Kaczor et al., 2015; Mozrzymas et al., 2011).

Current concepts about glycogen metabolism in muscle

Skeletal muscle contains glycogen at levels intermediate between brain and liver (Table 1). As in brain, glycogen in muscle undergoes continuous turnover and is not released as free glucose into the circulation. There is no evidence that muscle glycogen is metabolized in one cell to fuel activity in a neighboring cell, as has been proposed for glycogen in brain astrocytes. Skeletal muscle fibers types can be classified as Type 1 “red” or Type 2 “white”, with the red fibers containing more mitochondria and white fibers containing more glycogen (Nielsen et al., 2011). At the subcellular level, muscle glycogen is concentrated within and between individual myofibrils (the contractile elements and major ATP consuming sites) and between the outermost myofibril and myocyte cell membrane (Ortenblad and Nielsen, 2015). Glycogen is consumed during muscle activity and is re-synthesized from blood-borne glucose between muscle contractions(Nielsen et al., 2011; Shulman and Rothman, 2001). During very rapid or sustained muscle activity, glycogen consumption outpaces synthesis and is eventually depleted.

There is no evidence that muscle glycogen is metabolized in one cell to fuel activity in a neighboring cell, as has been proposed for glycogen in brain astrocytes. It is generally presumed that muscle glycogen is metabolized to augment circulating glucose to fuel the energy demand during very brief periods (10 - 40 milliseconds) of high ATP demand associated with individual contractions (Chin and Allen, 1997; Shulman and Rothman, 2001). However, glucose concentrations in muscle are in the millimolar range, far too high to be consumed in sub-second intervals, and with onset of exercise muscle glucose content increases rather than decreases, as a result of greater blood flow (Berger et al., 1975; Hamrin and Henriksson, 2008; Hamrin et al., 2011; Henriksson and Knol, 2005; MacLean et al., 1999; Rosdahl et al., 1993; Sahlin, 1990). Even in the absence of glucose, the 20-25 mM concentrations of phosphocreatine (PCr) in muscle are sufficient to maintain muscle ATP levels for at least several seconds (Baker et al., 2010; Bogdanis et al., 1996; Funk et al., 1989; Meyer, 1988) (see Box). Contraction can reduce the ability of blood to perfuse muscle, but this reduction is incomplete even with intense and sustained contraction (Lanza et al., 2005; McNeil et al., 2015; Wigmore et al., 2006).

Glycogen might alternatively be metabolized in rapidly exercising muscle to more quickly generate glucose-6-phosphate, the first intermediate of glycolysis. Maximal flux through glycogen phosphorylase substantially exceeds maximal flux through hexokinase in mammalian skeletal muscle (Suarez et al., 1997). An accelerated production of glucose-6-phosphate would not, however significantly increase the rate of glycolytic ATP production, because downstream phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase steps are rate-limiting (Kobayashi and Neely, 1979; Tanner et al., 2018).

[Text Box] Responses to graded energy demands: glycogen, phosphocreatine, and adenylate kinase.

Relationships between energy demand and energy supply can be divided into three stages.

(1) At rest or moderate exercise, glycolysis and mitochondrial oxidative phosphorylation together are able to generate ATP at a rate sufficient to meet demand. Mitochondrial oxidative phosphorylation is fueled by several substrates, including lipids, amino acids, ketone bodies, and lactate or pyruvate generated by glycolysis. Glycolysis can be fueled either by glucose or glycogen stores, but there is a net cost of one ATP per glucose moiety cycled on and off of glycogen polymer (Figure 1).

(2) With increasing energy demand, a point is reached at which the rate of mitochondrial oxidative phosphorylation is maximal, but the rate of glycolytic ATP production can further increase. The excess pyruvate resulting from the mismatch between glycolytic and oxidative metabolism is converted to lactate in order to regenerate the NAD^+ consumed by glycolysis, and the lactate is either exported to the blood stream or used locally when energy demand is lessened. Glycolysis can be fueled either by glucose or glycogen stores, but there is a net cost of one ATP per glucose moiety cycled on and off of glycogen polymer.

3) With extreme or very rapid onset energy demand, even maximal glycolytic flux cannot generate ATP rapidly enough to meet demand. Under these conditions, cells consume phosphocreatine to regenerate ATP through the creatine kinase reaction:

$$PCr + ADP + H^+ \leftrightarrow ATP + Cr$$

Given that the normal cytosolic ratio of ADP : ATP is low, this reaction has a proportionately far greater effect on lowering ADP than on increasing ATP. PCr metabolism also occurs during lower levels of energy demand to buffer local elevations in ADP and to facilitate intracellular dispersion of ATP and ADP equivalents (Wallimann, 1994). However, excitable tissues such as skeletal muscle and brain uniquely contain high levels of cytosolic PCr to permit a net consumption of PCr during intervals of rapid ATP hydrolysis.

Cells experiencing extreme levels of energy demand also employ the adenylate kinase reaction:

$$ADP + ADP \rightarrow ATP + AMP$$

This reaction likewise has a proportionately far greater effect on lowering ADP levels than on increasing ATP levels. The adenylate kinase pathway can be utilized for only limited intervals. because the AMP it generates is rapidly metabolized to inosine and ultimately ammonia, consequently depleting the total adenylate pool (ATP + ADP + AMP).

Most definitively, studies by Stephenson and colleagues using “skinned” (permeabilized) muscle fibers showed that fibers depleted of glycogen are unable to attain maximal contractile force even when perfused with glucose, PCr, and ATP (Stephenson et al., 1999), thus demonstrating that muscle glycogen must have functions other than simply a glucose store. These authors also noted that glycogen represents a sizeable sink for Pi in contracting muscle, and this provides a link between glycogen and muscle fatigue. Muscle fatigue is physiologically defined as a progressive decline in contractile force that largely recovers after rest (Allen et al., 2008). Fatiguing muscle also has reduced efficiency, i.e. produces less force per unit of metabolic substrate consumed (Broxterman et al., 2017; Gorostiaga et al., 2010).

Muscle fatigue correlates with glycogen depletion, (Allen et al., 2008; Ortenblad and Nielsen, 2015), but the primary factor causing muscle fatigue is ***not*** reduced ATP, but rather elevated inorganic phosphate (Pi) (Baker et al., 1993; Cooke et al., 1988; Miller et al., 1993). Elevated Pi concentrations actively suppress muscle activity in part by inhibiting the release of calcium from the sarcoplasmic reticulum that triggers muscle fiber contraction (Allen et al., 2002; Allen and Trajanovska, 2012). Thus the studies by Stephenson and colleagues suggest that Pi sequestration could be a primary function of glycogen metabolism in exercising muscle.

A thermodynamic role for glycogen metabolism

The capacity of cells to do work is determined not only by their levels of ATP (and other high-energy phosphate compounds), but also by the free energy of ATP hydrolysis ($\Delta G'_{ATP}$). The $\Delta G'_{ATP}$ in mammalian cells is usually near -55 kJ/mol. The more negative the value of $\Delta G'_{ATP}$, the more energy obtained from each molecule of ATP hydrolyzed to ADP. When the $\Delta G'_{ATP}$ rises (becomes less negative), ATP hydrolysis produces less energy. It is thus important for cells to maintain $\Delta G'_{ATP}$ in order to maximize both work capacity and work efficiency (i.e. work performed per substrate consumed). Omitting the terms for H^+, Mg^+ and H_2O for clarity (Alberty, 1969; Manchester, 1989), the simplified equation for ATP hydrolysis is:

$$ATP \rightarrow ADP + Pi$$

and the corresponding equation for the free energy ($\Delta G'$) for ATP hydrolysis is:

$$\Delta G'_{ATP} = \Delta G^{\circ\prime}_{ATP} + RT \ln ([ADP]\cdot[Pi] / [ATP])$$

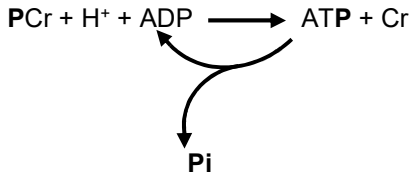
where $\Delta G^{0\prime}_{ATP}$ is the free energy of ATP hydrolysis under defined standard conditions (1M concentrations of all reactants and products), R is the gas constant, and T is temperature. (See (Iotti et al., 2005) for a more complete description). The equation clarifies that the amount of energy derived from ATP is influenced by the existing local concentrations of ADP and Pi. When the ratio of [ADP] · [Pi] / [ATP] is increased, the value of $\Delta G'$ becomes less negative and less energy is obtained.

During very rapid energy demand, the rate of ATP hydrolysis can exceed capacity for ATP regeneration. This affects cell energetics in two ways: it reduces ATP availability, and by elevating ADP and Pi it increases $\Delta G'_{ATP}$. Of these two, it is the increase in $\Delta G'_{ATP}$ that is most immediately significant. This is because the intracellular ATP concentration is normally 3 - 10 mM, and reductions in this concentration do not significantly limit the velocity of ATP-consuming enzymes unless they fall to near their Km ATP values, which is below 20 μM for both Na^+/K^+ ATPase and myosin ATPase (Hackney and Clark, 1985; Pilotelle-Bunner et al., 2008). By contrast, any elevation in $\Delta G'_{ATP}$ immediately reduces the amount of energy derived from ATP hydrolysis. This particularly affects the velocity of enzymes that operate near thermodynamic equilibrium with respect to $\Delta G'_{ATP}$, which include both Na^+/K^+ ATPase and myosin ATPase (Astumian, 2010; Ventura-Clapier et al., 1987; Wagoner and Dill, 2019; Wallimann, 1994).

The $\Delta G'_{ATP}$ is maintained in muscle and brain in part by creatine kinase reaction (see Box), which prevents elevations in ADP during rapid ATP hydrolysis while at the same time regenerating ATP.

$$PCr + ADP + H^+ \leftrightarrow ATP + Cr$$

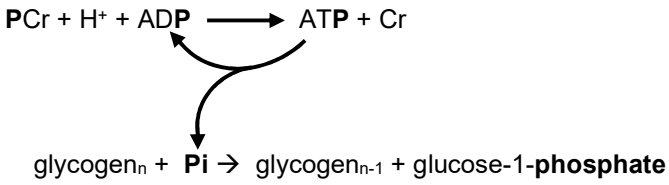
This reaction has a far greater relative effect on ADP levels (and thus $\Delta G'_{ATP}$) than on ATP levels because the ratio of ADP : ATP is normally very low (Wallimann, 1994). For this reason it has been proposed that the primary function of PCr under physiological conditions is to maintain $\Delta G'_{ATP}$ at sites of ATP hydrolysis by preventing local accumulation of ADP (Saks et al., 2007; Ventura-Clapier et al., 1987; Wallimann, 1994). However, the effects of PCr metabolism on $\Delta G'_{ATP}$ become more complex with rapid energy demand and net PCr consumption. Under these conditions, the phosphate moieties in PCr are released as Pi in proportion to net PCr consumption.



Elevated Pi, like elevated ADP, raises $\Delta G'_{ATP}$. This effect can be substantial not only in the immediate vicinities of ATP hydrolysis, but also at the whole-cell level. Whole-cell Pi concentrations rise from about 1 mM to above 5 mM during net PCr consumption (Bogdanis et al., 1996; Bogdanis et al., 1998; Gaitanos et al., 1993; Sahlin et al., 1997), and elevated cytosolic Pi is the primary biochemical cause of muscle fatigue, as noted above. Crucially, the glycogen phosphorylase reaction serves to attenuate the rise in Pi:

Pi + $glycogen_n$ → $glycogen_{n-1}$ + glucose-1-**phosphate**

PCr and glycogen can thus act in tandem to preserve $\Delta G'_{ATP}$ by limiting the rise of both ADP and Pi that would otherwise occur during rapid ATP hydrolysis.



The attenuation of Pi elevation by glycogen metabolism can thus counter the effect of Pi on $\Delta G'_{ATP}$. As with PCr metabolism (Saks et al., 2007; Ventura-Clapier et al., 1987; Wallimann, 1994), this effect of glycogen metabolism is likely most important in the immediate vicinities of ATP hydrolysis. Here the coupled effects of PCr and glycogen metabolism can supplement diffusion to attenuate local elevations in ADP and Pi, respectively. The effect of Pi sequestration on $\Delta G'_{ATP}$ might be spatially and temporally limited if the Pi bound as glucose-1-phosphate (and its downstream metabolites) were rapidly liberated back to the cytosol. However, measurements from actively exercising muscle show that a substantial fraction of the Pi remains sequestered as millimolar concentrations glucose-6-phosphate, and to a lesser

extent as other glycolytic intermediates, until exercise ends and PCr is resynthesized (Bendahan et al., 1990; Bendahan et al., 1992; Bogdanis et al., 1996; Bogdanis et al., 1998; Gaitanos et al., 1993; Kappenstein et al., 2013).

Pi sequestration in glycolytic phosphoesters could in principle also be accomplished by metabolizing glucose, rather than glycogen. This does not occur in the first, hexokinase step of glycolysis, but does occur after accounting for subsequent ATP re-synthesis, as shown below in the summed reactions of glycolysis.

glucose + ATP $\xrightarrow{\text{hexokinase}}$ glucose-6-phosphate + **ADP**

glucose-6-phosphate + 3ADP + **2Pi** → 3ATP + 2 lactate (glycolysis)

However, direct comparison of the net effects using glycogen vs. glucose as the substrate shows that glycogen metabolism removes a net one more Pi molecule per glucose moiety consumed, and produces one less molecule of ADP.

glycogen_n + **Pi** $\xrightarrow{\text{glycogen phosphorylase}}$ glycogen_{n-1} + glucose-1-phosphate ←→ glucose-6-phosphate

glucose-6-phosphate + 3ADP + **2Pi** → 3ATP + 2 lactate (glycolysis)

Thus, relative to glucose, glycogen utilization maintains $\Delta G'_{ATP}$ by removing Pi from the cytosol in its first step, by removing a net of 1 more Pi through glycolysis, and by producing one less ADP. It may also be significant that glucose-6-phosphate can be produced from glycogen much faster than it can be produced from glucose (Suarez et al., 1997).

Biological importance of glycogen metabolism for maintaining $\Delta G'_{ATP}$.

Published measurements of metabolite concentration changes in exercising muscle permit a quantitative appraisal of the effect of glycogen utilization on $\Delta G'_{ATP}$ at the whole-cell level. As shown in Table 2, the binding of phosphate in glycogen-derived metabolites can be estimated to reduce the relative change in $\Delta G'_{ATP}$ by roughly 40% (from 9.2 to 5.6 kJ/mol) during muscle exertion that nearly depletes PCr. This estimate is based on bulk tissue measurements, which necessarily underestimate the magnitude of subcellular changes. Pi elevations in the immediate vicinity of rapid ATP - consuming processes are likely faster and larger than estimated by the bulk tissue measurements, with a proportionately greater impact of Pi sequestration on subcellular $\Delta G'_{ATP}$ values. To place this in context, even very small changes in work capacity can translate to significant differences in competitive advantage; for example, the difference in the 100 meter sprint times between Olympic gold medalists and fourth place finishers is typically less than 1.5%. The increased efficiency provided by glycogen Pi sequestration also predicts that proportionately less substrate need be consumed to perform the same amount of work.

The thermodynamic effect of glycogen metabolism can is also evidenced in McArdle disease, in which genetic deficiency in muscle glycogen phosphorylase (PGYM) prevents mobilization of skeletal

muscle glycogen (Nogales-Gadea et al., 2016). Individuals with McArdle disease have no difficulty with moderate levels of exercise (such as walking), but develop early onset muscle fatigue with strenuous exercise, followed by muscle cramps (sustained, electrically silent contractions) and myocyte disruption if exercise is continued. The ATP concentration in exercising muscle of these individuals does not fall significantly lower than in normal subjects (or in resting muscle), but Pi concentrations rise faster and further than in normal muscle (Bendahan et al., 1992; Lofberg et al., 2001; Malucelli et al., 2011). This rise can be directly attributed to the absence of phosphate binding in glycogen-derived intermediates because, in contrast to normal muscle, the rise in Pi is nearly equimolar with the fall in PCr, and there is no increase in phosphomonoesters (Bendahan et al., 1990; Bendahan et al., 1992; Rowland et al., 1965). These observations also indicate that glycogen is by far the primary mechanism used by myocytes to buffer Pi elevations.

The overall efficiency of muscle contraction – i.e. the work produced relative to the energy substrate consumed – is reduced in McArdle disease (O'Dochartaigh et al., 2004), and the calculated $\Delta G'_{ATP}$ at end-exercise is higher (less negative) than in normal muscle despite being more negative at rest (Malucelli et al., 2011). McArdle disease muscle also forms inosine and ammonia with even moderate exercise (Kazemi-Esfarjani et al., 2002), indicative of adenylate kinase activation (see Box). While the adenylate kinase reaction generates only small increases in ATP, it produces proportionately far greater reductions in ADP levels. Activation of the adenylate kinase reaction thus illustrates the biological importance of maintaining muscle $\Delta G'_{ATP}$ because it comes with the unsustainable cost of depleting the total adenylate pool (ATP + ADP + AMP).

An additional feature of McArdle disease has long been unexplained: the absence of any net increase in lactate or H^+ production (aerobic glycolysis) in exercising muscle (Bendahan et al., 1992; Kazemi-Esfarjani et al., 2002; Lofberg et al., 2001), despite adequate glucose delivery (Berger et al., 1975; Hamrin and Henriksson, 2008; Hamrin et al., 2011; Henriksson and Knol, 2005; MacLean et al., 1999; Rosdahl et al., 1993) and even with induced hyperglycemia (Haller and Vissing, 2002; Lewis et al., 1985). We propose that this feature may also be a consequence of deficient Pi sequestration in exercising McArdle disease muscle. Given that elevated Pi suppresses muscle contraction (Allen et al., 2002; Allen and Trajanovska, 2012), the failure to increase aerobic glycolysis may reflect a lack of metabolic demand induced by elevated Pi, rather than incapacity of the muscle fibers to use glucose. This concept is supported by the strikingly attenuated increase in blood flow in exercising McArdle disease muscle (Jehenson et al., 1995), an observation that is difficult to explain except by reduced metabolic demand. Of interest, there is evidence for increased epilepsy incidence in McArdle disease (Mancuso et al., 2011), though otherwise there is no indication of brain dysfunction (presumably because astrocytes normally express the brain form of glycogen phosphorylase, PGYB, in addition to the muscle PGYM form).

The importance of maintaining $\Delta G'_{ATP}$ is most evident under extreme conditions such as intense exercise and McArdle disease, but it is relevant at all levels of energy metabolism to the extent that processes operating at an elevated (less negative) $\Delta G'_{ATP}$ must consume more substrate to achieve

equal amounts of work. Moreover, as demonstrated with the skinned muscle fiber preparation, maximal force cannot be achieved in the absence of glycogen even when glucose, PCr, and ATP are available (Stephenson et al., 1999). Myosin ATPase is near thermodynamic equilibrium, meaning that the probability of forward movement of the actin/myosin motor is at all times influenced by existing the $\Delta G'_{ATP}$ (Astumian, 2010). This is similarly true of other ATPases, including the Na^+/K^+ ATPases that drive transmembrane ion transport in brain and other tissues (Ventura-Clapier et al., 1987; Wagoner and Dill, 2019; Wallimann, 1994). By contrast, the functions of these enzymes are minimally affected by changes in ATP concentration that occur under physiological conditions.

Thermodynamic aspects of glycogen metabolism in brain

The functional effects of $\Delta G'ATP$ are more difficult to quantify in brain than in muscle, where fiber contraction provides a convenient read-out of work capacity, but evidence that glycogen metabolism is also required for optimal bioenergetic efficiency in brain comes from observations suggesting glycolytic super-compensation when glycogen metabolism is blocked. Glycolytic super-compensation describes an increase in glucose utilization above what is needed to compensate for lack of ATP production from glycogen (Walls et al., 2009). Studies by Dienel and colleagues found that activity - induced increase in glucose utilization in rat sensory cortex was increased by 1.7 – 2.9 fold by an inhibitor of glycogen phosphorylase (Dienel et al., 2007). While the proportion of glucose consumed by astrocytes vs. neurons is uncertain, this result suggests a reduction in brain energetic efficiency in the absence of glycogen, analogous to the increased use of substrate in McArdle disease muscle (Malucelli et al., 2011; O'Dochartaigh et al., 2004).

Brain, like muscle, responds to rapid fluctuations in energy demand in part by increased turnover and net consumption of PCr (Jost et al., 2002; Mora et al., 1991). PCr in brain is enriched in astrocytes, along with glycogen. All cell types contain the ubiquitous mitochondrial creatine kinase, but only certain tissues are enriched in cytosolic creatine kinase. These are skeletal muscle, which expresses the CKm isoform; brain, which expresses the CKb isoform; and heart, which expresses both isoforms. In brain, CKb is expressed at much higher levels in astrocytes than in neurons (Lowe et al., 2013; Manos et al., 1991; Zhang et al., 2014). The co-enrichment of cytosolic PCr and glycogen in astrocytes, as in myocytes, thereby permits their coupled effects on $\Delta G'_{ATP}$ during intervals of rapid energy demand.

There has been a long-standing interest in the fact that glycogen is primarily localized to astrocytes even though neurons account for most brain energy expenditure (DiNuzzo et al., 2017; Harris et al., 2012). There has been less focus on why astrocytes also contain more PCr than neurons, but the answer to both questions may stem from the unique need for astrocytes to react to abrupt local changes in local extracellular milleu during neuronal activity. Glutamate, the primary excitatory neurotransmitter in mammalian brain, is removed from the extracellular space almost exclusively by re-uptake into astrocytes (Anderson and Swanson, 2000). Astrocytes maintain a glutamate concentration gradient of roughly 10,000 : 1 across their cell membranes through the activity of sodium-dependent glutamate transporters,

which consume more than 2 ATP per molecule of glutamate transported against this gradient. Neuronal post-synaptic depolarizations and action potentials also release K^+ into small extracellular compartments, requiring immediate local, energy dependent K^+ uptake by astrocytes, which has been calculated to require an even greater astrocytic energy expenditure (DiNuzzo et al., 2017). Analogous to skeletal muscle, these demands on astrocytes are variable and episodic, particularly in their thin, synapse - ensheathing distal processes. Neuronal electrical activity is fueled by the membrane potential that is maintained over the entire surface area of the cell, and the energy demand induced by intervals of neuronal activity are thus distributed both spatially and temporally. By contrast, the uptake of glutamate and K^+ by astrocytes is only minimally electrogenic (Anderson and Swanson, 2000), and requires immediate local activation of Na^+/K^+ ATPase in order to maintain the transmembrane Na^+ gradient that drives these uptake processes. It is thus significant that glycogen phosphorylase has been identified in a macromolecular assembly with the astrocyte glutamate transporter, GLT-1 (Genda et al., 2011), and that astrocytes in which glycogenolysis is pharmacologically inhibited show reduced capacity for rapid uptake of K^+ even when glucose is readily available (Xu et al., 2013).

Summary

There is little question that glycogen can serve as an emergency energy store in brain, that astrocyte glycogen utilization can spare glucose for neuronal use, and that lactate generated by astrocyte glycogenolysis can subsequently be used by neurons (or other cells) for oxidative energy metabolism. There is similarly little question that glycogen in muscle can serve as a local glucose reserve. However, these functions do not adequately account for several other aspects of glycogen metabolism in brain and muscle, specifically that (1) in both tissues, glycogen is metabolized even when glucose is available, despite the net ATP cost of using glycogen; (2) in both tissues, evidence suggests a reduced efficiency in the absence of glycogen; (3) in brain, glycogen is localized primarily to astrocytes along with elevated levels of PCr; and (4) muscle deficient in glycogen phosphorylase (McArdle disease) fails to increase glycolytic metabolism or local blood flow, but instead activates the adenylate kinase reaction to lower ADP levels. Each of these aspects of glycogen metabolism can be understood by the thermodynamic considerations presented here. The thermodynamic view holds that it is equally or more important for cells to maintain the amount of energy derived from ATP hydrolysis as to maintain ATP concentrations, because cells with less negative $\Delta G'_{ATP}$ have reduced maximal work capacity and must consume more ATP to do equivalent work. $\Delta G'_{ATP}$ is increased when ADP or Pi increase. ADP and Pi concentrations increase in cells (or in subcellular domains) that must transiently utilize ATP faster that it can be regenerated. The coupled metabolism of PCr and glycogen buffers elevations in ADP and Pi and thereby maintains $\Delta G'_{ATP}$. This effect may be particularly important in subcellular domains where simple diffusion is insufficient to quickly dissipate local elevations induce during rapid ATP hydrolysis.

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Table 1. Tissue glycogen concentrations

Tissue	Glycogen content (mg / g)	(μmol / g)	Citation
Liver	29.02 ± 2.4	181.0 ± 15.0	(Vissing et al., 1989)
Liver	41.39 ± 5.28*	258.2 ± 32.9*	(Kusunoki et al., 2002)
Muscle (gastrocnemius-plantaris-soleus complex)	5.70 ± 0.24[a]	35.6 ± 1.5[a]	(Baker et al., 2005)
Muscle (white gastrocnemius)	5.56 ± 0.69	34.7 ± 4.3	(Garetto et al., 1984)
Muscle (white gastrocnemius)	6.73 ± 0.16	42.0 ± 1.0	(Vissing et al., 1989)
Brain (cortex)	0.65 ± 0.03	4.1 ± 0.2	(Sagar et al., 1987)
Brain (cortex)	2.01 ± 0.26	12.5 ± 1.6	(Cruz and Dienel, 2002)
Brain (whole brain)	1.98 ± 0.20	12.3 ± 1.3	(Oe et al., 2016)
Heart	4.33 ± 0.32	27.0 ± 2.0	(Vissing et al., 1989)
Heart	3.99 ± 0.22	24.9 ± 1.4	(Conlee et al., 1989)
Kidney	0.06 ± 0.01	0.4 ± 0.1	(Khandelwal et al., 1979)
Kidney	0.03 ± 0.01*	0.2 ± 0.1*	(Nannipieri et al., 2001)

Glycogen in brain is restricted primarily to astrocytes, which comprise less than 25% of brain volume (Gundersen et al., 2015).

Data are from adult rat or mouse under rest, non-fasted conditions. Values are means ± SEM or means ± SD (*).

[a] Reported as mmol/kg dry weight; converted here using values of 160.3 mg /mmol as molecular weight of glycogen (Wu et al., 2019) and 76% as the water content of muscle.

Table 2. Estimated effect of Pi sequestration by glycogen metabolism on exercise -induced changes in whole-cell $\Delta G'_{ATP}$

	Glycogen (mM glucose equivalents)	PCr (mM)	ATP (mM)	ADP (mM)	Pi (mM)	$\Delta G'_{ATP}$ (kJ/mol)	Change in $\Delta G'_{ATP}$ relative to rest
At rest	114	26.32	9.45	1.19	1.01	- 53.6	- - -
End-exercise	80	4.41	6.86	1.37	5.67	- 48.0	5.6 kJ/mol (10.5%)
(Calculated) end-exercise in the absence of glycogen	- - -	4.41	6.86	1.37	22.9	- 44.4	9.2 kJ/mol (17.2%)

The rise in muscle Pi during strenuous exercise reduces the energy ($\Delta G'_{ATP}$) that can be obtained from ATP hydrolysis. The rise in Pi and the corresponding rise in $\Delta G'_{ATP}$ increase in the absence of glycogen because Pi is normally sequestered in glycogen-derived hexose monophosphates and other glycolytic intermediates. Metabolite values at rest and end-exercise are from biopsy studies of human quadriceps muscle: glycogen, PCr, ATP, and Pi from (Bogdanis et al., 1996), and ADP from (Sahlin et al., 1997). Similar values are reported in other studies (Bogdanis et al., 1998; Gaitanos et al., 1993). It is assumed for this estimation that in the absence of glycogen, PCr consumption produces equimolar amounts of Pi, and that rats of PCr consumption and ATP turnover are unaffected. In actual muscle, rates of PCr consumption and ATP turnover would be suppressed by increased Pi (as occurs in McArdle disease). $\Delta G'_{ATP}$ values are calculated as $\Delta G°'_{ATP}$ + RT ln ([ADP]·[Pi] / [ATP]), using -30.5 kJ / mol for $\Delta G°'_{ATP}$ and using 2.58 kJ / mol for RT at 37° C. For simplicity, the calculated $\Delta G'_{ATP}$ values do not account for the relatively smaller effects Mg^{2+} and H^{+} binding to the metabolites (Iotti et al., 2005). The calculations also do not account for possible changes in the ratios of free : bound metabolites during exercise.

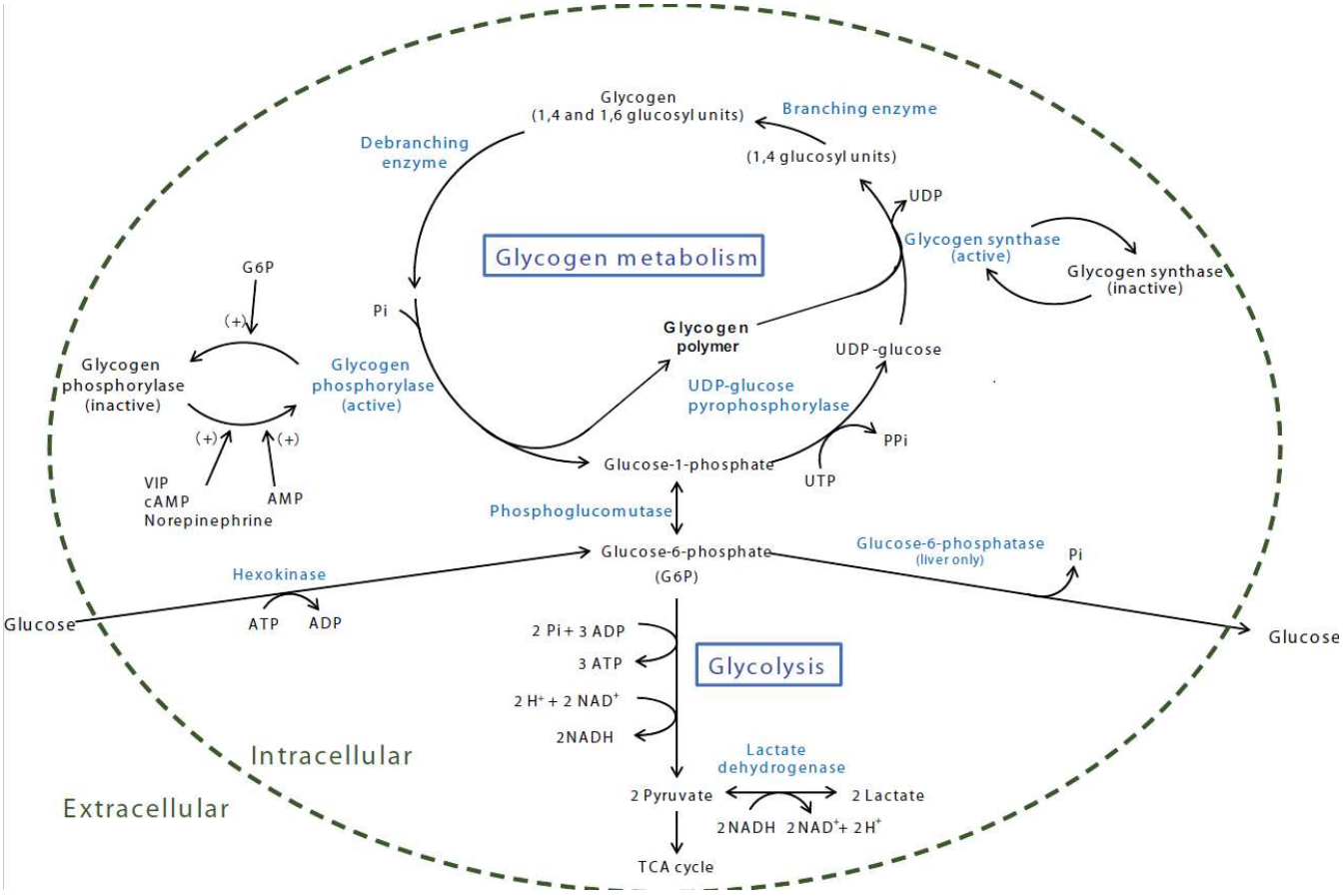

Figure 1. Bioenergetics and regulation of glycogen metabolism. Glycogen synthase extends an existing glucosan chain of α-1, 4-glycosidic linkages using UDP glucose as substrate. Glycogen branching enzyme subsequently forms α-1, 6-glycosidic bonds to create branch points. Glycogen degradation is mediated by debranching enzyme and glycogen phosphorylase. Glycogen phosphorylase is regulated allosterically in response to neurotransmitters and hormones, by changes in energy state (AMP, glucose-6-phosphate, and others), and by second messengers such as cAMP. The immediate product of glycogen phosphorylase is glucose 1-phosphate, which is freely converted to glucose-6-phosphate. Hepatocytes (but not other cell types) can rapidly dephosphorylate glucose-6-phosphate to generate free glucose for export. There is a net cost of one ATP per molecule of glucose-6-phosphate that is cycled onto and off of the glycogen polymer, as 2 ATP equivalents are consumed in forming UDP glucose from glucose-1-phosphate and 1 ATP equivalent is gained back at the formation of glucose-1-phosphate from a glucose residue and inorganic phosphate (Pi) at the glycogen phosphorylase step.

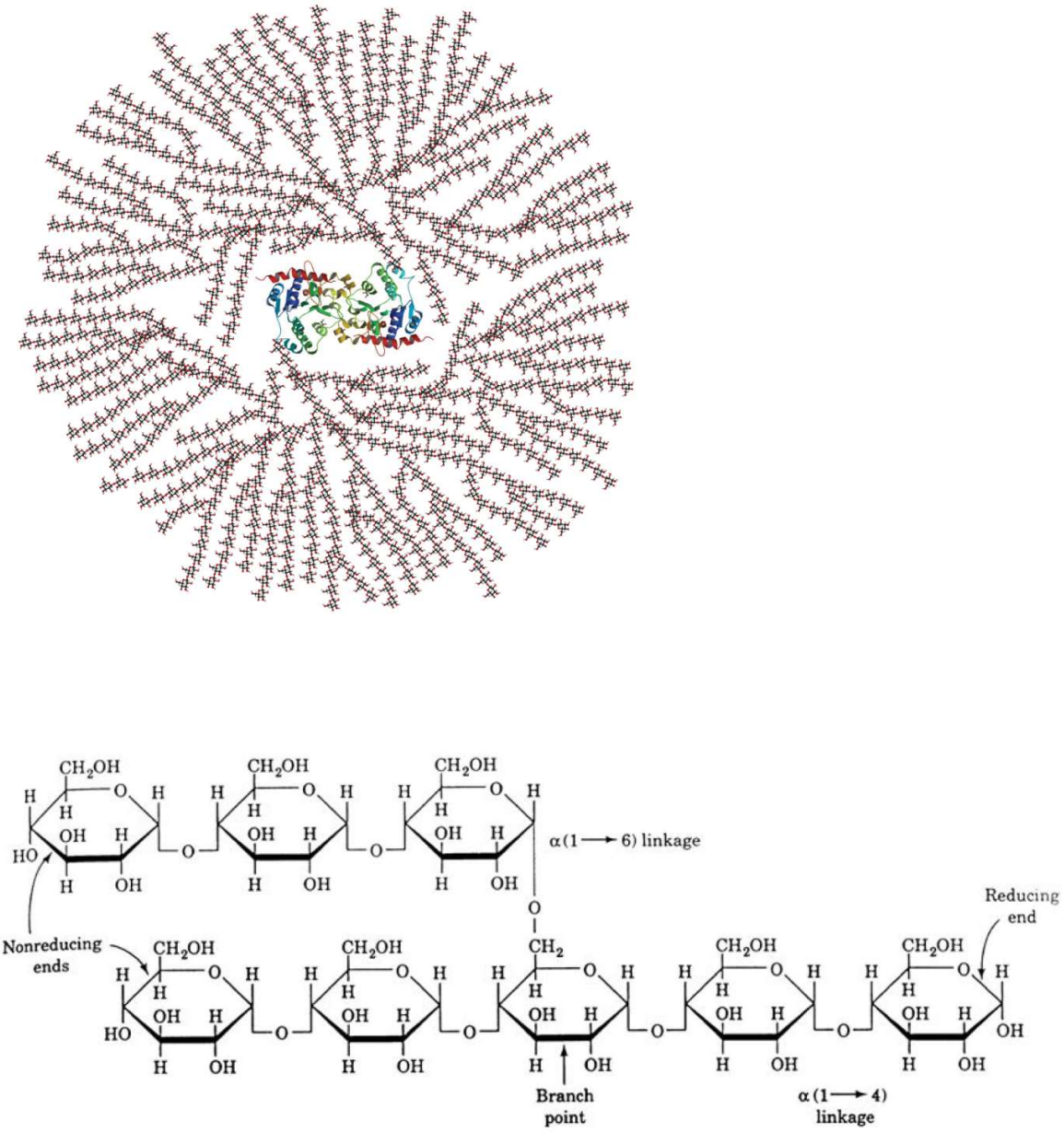

Figure 2. Schematic two-dimensional cross-sectional view of glycogen. A core protein of glycogenin is surrounded by branches of glucose units. The entire globular granule may contain around 30,000 glucose units. The individual glucose moieties of glycogen are linked by α-1, 4 -glycosidic bonds, with branch points at approximately every 10 - 14 glucose residues linked by α-1, 6-glycosidic bonds. The exposed ends of all glycogen chains are non-reducing. Image from (Haggstrom, 2014) .

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