The structure of brain glycogen phosphorylase-from allosteric regulation mechanisms to clinical perspectives

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The structure of brain glycogen phosphorylase: from allosteric regulation mechanisms to clinical perspectives

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Article type : Structural Snapshot

ABSTRACT

Glycogen phosphorylase (GP) is the key enzyme that regulates glycogen mobilization in cells. GP is a complex allosteric enzyme that comprises a family of three isozymes: muscle GP (mGP), liver GP (lGP) and brain GP (bGP). Although the three isozymes display high similarity and catalyze the same reaction, they differ in their sensitivity to the allosteric activator AMP. Moreover, inactivating mutations in mGP and lGP have been known to be associated with glycogen storage diseases (McArdle and Hers disease, respectively).

The determination, decades ago, of the structure of mGP and lGP have allowed to better understand the allosteric regulation of these two isoforms and the development of specific inhibitors. Despite its important role in brain glycogen metabolism, the structure of the brain GP had remained elusive.

Here, we provide an overview of the human brain GP structure and its relationship with the two other members of this key family of the metabolic enzymes. We also summarize how this structure provides valuable information to understand the regulation of bGP and to design specific ligands of potential pharmacological interest.

Keywords: glycogen metabolism, phosphorylase, allosteric regulation, crystal structure

Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; FMN, flavin mononucleotide; GP, glycogen phosphorylase; bGP, brain glycogen phosphorylase; lGP, liver glycogen phosphorylase; mGP, muscle glycogen phosphorylase; PEG, polyethylene glycol; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle.

INTRODUCTION

Glycogen, the main carbohydrate store in mammalian cells, provides glucose for energy metabolism pathways including the glycolysis as well as the TCA cycle and the pentose phosphate pathway (1). humans glycogen performs various functions depending on its location. In liver, it is metabolized into glucose and released into the systemic circulation to regulate the glycemia. In muscles, this polymer provides energy for the muscular contraction (2). In the brain, glycogen has long been considered as an emergency store of glucose, mobilized to protect neurons against hypoglycemia and hypoxic stress (3, 4). Yet, it has become clear that astrocytic glycogen sustains neuronal activity and is an important component of brain physiology (5). In particular, recent findings have provided evidences that glycogen mobilization and glycogen-derived lactate are critical for high cognitive processes such as learning and memory consolidation (6, 7). Glycogen phosphorylase (GP) is the key enzyme of glycogen mobilization (glycogenolysis) in cells. In humans, this enzyme is found as three isoforms named after the organ where they predominate : liver (lGP), muscle (mGP) and brain GP (bGP). These three isoenzymes are tightly regulated through both the binding of allosteric effectors and the phosphorylation of the Ser14, in response to intracellular and extracellular energy demand respectively. Despite their high sequence identity, the three isoforms of GP can be distinguished by

their structural, regulatory and functional properties. On one hand, lGP is mostly regulated through phosphorylation, allowing the use of liver glycogen in response to hypo and hyperglycemic hormones. On the other hand, mGP and bGP respond to the binding of allosteric effectors, thus controlling glycogenolysis according to local energy needs. Nevertheless, mGP and bGP differ in their regulation by AMP and phosphorylation. mGP strongly and cooperatively responds to AMP activation and to phosphorylation, whereas bGP is strongly but non-cooperatively activated by AMP. Therefore, the different regulation of glycogen metabolism in liver, muscles and brain dramatically relies on the regulation of each GP isoenzyme (2). Crystallographic determination of the structure of these three enzymes led to a better understanding of the structural basis of these regulations and the development of drugs (8–11). Here, we review conclusions from our recent determination of the structure of human bGP (PDB ID: 5IKO and 5IKP). We also discuss insights provided by this structure concerning the metabolism of glycogen in the brain and present the perspectives for future research and clinical therapy (12).

DETERMINATION OF THE bGP STRUCTURE

Even with its large size, oligomeric organization and allosteric regulation the crystal structure of mGP(from rabbit muscle)was obtained in early 70s. Many structures of this isoenzyme were then obtained in presence of different allosteric effectors and/or drugs, allowing a better understanding of the allosteric regulation of mGP (Figure 1) (8, 13, 14). Later, in 2000, the crystallization and structural determination of lGP put in light the structural bases for the different regulation of these isoenzymes (Figure 1) (9). Yet, the crystal structure of the brain form of GP remained unknown.

Contrary to mGP or lGP, bGP appears to be expressed at relatively low level, in brain, and is prone to aggregation. Consequently, its expression in recombinant systems and its purification has been a difficult task. To increase its expression and ensure the structural/functional integrity of the protein, we expressed the recombinant human bGP in *E. Coli* bacteria expressing the GroEL/GroES chaperonin complex. The co-expression of GroEL/GroES complex significantly improved the correct folding of bGP. Thanks to this method we could obtain relatively large amount of recombinant bGP,

displaying the full biochemical and enzymatic characteristics previously described for this isozyme (Figure 1) (12).

We obtained two crystal structures of human bGP: one in complex with PEG 400 (used as a precipitant in the crystallization buffer)(PDB ID: 5IKO) and one in complex with AMP (PDB ID: 5IKO), obtained by soaking off the initial crystal in a solution containing a high concentration of AMP. Similar to what was previously described for mGP and lGP, bGP is found as a homodimer and shares a similar global structure with the two other isozymes (RMS Deviation < 1 Angstrom between the different isoenzymes) (Figure 1). GPs can be depicted as having two faces : (i) the regulatory face which comprises the regulatory elements including the AMP binding site and the phosphorylation peptide (containing the Ser14); (ii) the catalytic face composed of the active site of the enzyme (8, 9).

As stated above, GP enzymes are allosteric enzymes, found in at least two states in equilibrium : an active state (R-state) and an inactive state (T-state) (2). Comparison of the structures of the three isoenzymes showed that our structures of bGP are highly similar to the active state of mGP, more particularly at the dimer interface. One main component of the dimer interface in GP isoenzymes are the Tower helices (helix 7 of each monomer). These two helices display an anti-parallel association and govern the dimerization as well as the activation of the enzyme (8). They present a typical crossover angle of 75° and 45° in active mGP and lGP respectively, related to the activation process of each isoform (Figure 2) (8, 9). In mGP a dramatic rearrangement of the dimer interface, including the Tower helices rotation by 50 °, characterizes the activation of the enzyme and is allowed by a flexible dimer interface. The remodeling of the overall relationship of the dimer leads to the opening of the catalytic site and, thus, the activation of the enzyme (Figure 2) (8). On the contrary, in lGP activation is promoted by a compaction of the catalytic core of the enzyme, including the Tower helices. However, due to the rigid body of the lGP dimer, no rotation of the Tower helices is observed during activation (Figure 2) (9). Our bGP structures were characterized by a highly flexible dimer interface and the Tower helices displayed a cross over angle of 85° (Figure 2). Consequently, these

observations suggest that activation of bGP occurs through a rearrangement of the dimer interface, similar to what is observed for mGP (12).

STRUCTURAL BASES OF bGP REGULATION BY AMP AND PHOSPHORYLATION

Different signals regulate GP activation and inactivation. On one hand, the phosphorylation of the Ser14 in response to extracellular energy demand and the glycogenolytic cascade (phosphorylation cascade) leads to the activation of the enzyme (Figure 3). On the other hand, these enzymes are also regulated through the binding of allosteric effectors, including AMP, ATP or glucose-6-phosphate, and thus respond to local energy needs (Figure 3) (2).

Contrary to lGP or mGP, bGP is poorly activated by phosphorylation of Ser14. Although we did not obtain crystals for the phosphorylated bGP, our structural data provided molecular insights on the regulation of bGP by phosphorylation. Indeed, in mGP and lGP, phosphorylation or AMP binding lead to the stabilization of the phosphorylation peptide, through its interaction with the dimer interface (8, 9). Even though we obtained the structure of the active bGP, no electron density was observed for the phosphorylation peptide (Figure 3). This could be due to amino acids substitution between lGP, mGP and bGP (such as Glu22 in mGP and lGP substituted by a Gly in bGP, or mGP and lGP Lys28 replaced by an Arg in bGP), located at the dimer interface, which might reduce the stabilization of the phosphorylation peptide in bGP, leading to the absence of electron density as well as the limited sensitivity of bGP to phosphorylation.

On the contrary, AMP is a potent activator of bGP. It interacts with the enzyme in the AMP binding site, located at the dimer interface. Upon binding to the AMP binding site, AMP induces structural changes at the dimer interface, allowing the conversion of the inactive enzyme to its active state. GP AMP binding site is composed of several secondary structures : helices 2 and 8 as well as the β4/ β5 loop and the adenine loop from one subunit, and the Cap' loop from the other subunit (Figure 3 and 4) (8, 9, 14).

The AMP binding site can be divided into three regions which bind the phosphate moiety, the ribose moiety and the adenine moiety of AMP respectively (8, 14). In our structure, the phosphate group of the allosteric activator governs the binding of AMP. Indeed, three of the four hydrogen bonds involved in the binding of AMP are established, between the phosphate group of AMP and Tyr196, located in the β4/ β5 loop, and between Arg309 and Arg310 which belong to the helix 8. Interestingly, the interaction between AMP and Tyr196 is unique to the brain isoform of GP. Indeed, this amino acid is substituted by a Phe in the muscle and liver form (Figure 4). This particular bond allows a strong interaction between the dimer interface and the activator. In association with the high flexibility of bGP dimer interface, the interaction between AMP and Tyr196 could participate to the important sensitivity of the bGP to its allosteric activator compare to mGP (12).

In addition, in bGP, AMP also establishes a hydrogen bond with the cap' loop. However, no strong interactions were observed between the helix 2 and the allosteric activator. The helix 2 extends across the width of the protein, ensures the connection between the two AMP binding site and is thus involved in the cooperative binding of AMP. In mGP, helix 2 strongly interacts with AMP through the establishment of a hydrogen bond between Tyr75 and the phosphate moiety of AMP (Figure 4) (8, 14). However, in bGP, no such bond is observed. Tyr75 only establishes a co-planar stacking with the adenosine part of AMP. Consequently, the loss of a strong interaction between AMP and helix 2 might participate in the non-cooperative binding of AMP in bGP. Moreover, the particular orientation of Tyr75 in bGP precludes the stabilization of the adenine loop, a key secondary structure involved in the binding of AMP in mGP, which is suspected to be responsible for the AMP/IMP specificity and the cooperative binding of AMP (Figure 4) (9, 14). The absence of contacts between the adenine loop and AMP in bGP may thus also participate in the non-cooperative binding of AMP and could impact the sensitivity and regulation of bGP toward AMP analogous such as IMP.

IMPLICATION OF bGP STRUCTURES FOR THE UNDERSTANDING OF GLYCOGEN METABOLISM IN BRAIN AND HEART

In the brain, bGP is predominantly found in astrocytes, which contain most of the brain glycogen store, and to less extent in the neurons (15, 16). However, while neurons only express the brain form of GP, astrocytes co-express both the brain and the muscle isoforms (17). The expression of two isoforms in the brain suggests that each of these enzymes perform one or more specific functions which are closely related to their regulation. Therefore, it was previously suggested that mGP, which is sensitive to phosphorylation, controls the brain glycogenolysis in response to the extracellular signals such as neurotransmitters. On the contrary, bGP is more sensitive to AMP activation and thus allow the use of the glycogen store in response to local energy need (Figure 3) (18). Our structural data now gave molecular insights about the different sensitivity of bGP and mGP to their activators.

However, very little is known about the inhibition of brain glycogenolysis by allosteric inhibitors. Indeed, the availability of a cellular glycogen store is physiologically meaningful only to respond to a temporary and intermittent demand of glucose and/or glycosyl residues. Consequently, phases of high energy demand leading to the use of glycogen store are followed by phases of limited demand allowing the replenishment of the stores (1). In cells, the restoration of glycogen store in cells relies on the inhibition of GP and the allosteric activation of the glycogen synthase (GS), the cytosolic enzyme responsible for glycogen synthesis (1). GP enzymes present three allosteric sites involved in the inhibition of the enzyme : the catalytic site, the inhibitor site (or purine site), involved in the binding of purine such as caffeine or FMN, and the AMP binding site (2). Despite the catalytic site and the purine site are highly conserved among GPs, distinguishing features observed between mGP and bGP AMP binding sites could influence the inhibition of the glycogen mobilization in the brain. Indeed, the AMP binding site is also involved in the binding of ATP, ADP and glucose-6-phosphate, three allosteric inhibitors of GP enzymes. These three effectors stabilize the inactive conformation of GP and inhibit the phosphorylated enzyme (2). So far, only the crystal structure of mGP in complex with glucose-6-phosphate has been determined (PDB ID : 1GPY). In this structure, glucose-6-phosphate establishes different hydrogen bonds and Van der Waals interactions with residues located

in the AMP binding site, including Arg309, Arg310, Gln71 which are involved in the binding of AMP, as well as several residues located at the dimer interface including for instance Arg193, Asp227 as well as Arg242. Interestingly, Phe196 further stabilize glucose-6-phosphate in mGP (19). The substitution of Phe196 for a Tyr in bGP might thus influence the binding of glucose-6-phosphate in the AMP binding site, through the formation of hydrogen bond between this residue and the phosphate group of glucose-6-phosphate. Furthermore, in bGP, Tyr196 might also be involved in the binding of ATP and ADP, leading to a specific regulation of bGP by these allosteric inhibitors. The potential different behaviors of bGP and mGP toward ATP, ADP and glucose-6-phosphate could thus help to better understand the inhibition of glycogenolysis in the brain.

Besides, the different regulation of bGP and mGP by allosteric inhibitors would be particularly relevant in the brain. Indeed, energy metabolism differs between neurons and astrocytes. While the production of ATP mainly relies on the mitochondria in neurons, energy metabolism is predominantly carried out through glycolysis in astrocytes. Therefore, in neurons, glycogen mainly feeds the pentose phosphate pathway and provides protection during oxidative stress. However, glucose-1-phosphate derived from glycogen can be further metabolized into glucose-6-phosphate and supply both the pentose phosphate pathway and the glycolysis in astrocytes (20). Giving the different expression of bGP and mGP in brain cells, specific regulation of these two isoforms by glucose-6-phosphate could thus influence the functions of glycogen. In addition, ATP also acts as a neurotransmitter in the brain. It has been shown to induce the phosphorylation as well as the activation of GP, hence performing a dual regulation of glycogen metabolism (21). The different regulation of mGP and bGP by ATP could participate to the tight regulation of glycogen metabolism in response to neuronal activity.

Interestingly, bGP is also strongly expressed in the heart, at a level similar to what is found in the brain, and is co-expressed with mGP, suggesting that bGP must be important for cardiac function (22). It is now obvious that expression of bGP in the brain, is associated with a local use of glycogen store to provide glucose for the cell itself (18). It is likely that bGP exert also this function in the heart, another electrically excitable tissue (22). The molecular insight raised by bGP structure will

thus also help to better understand glycogen metabolism in the heart, which appears to differ from what we observe in skeletal muscles.

RATIONAL DRUG DESIGN AND THERAPEUTIC PERSPECTIVES

Glycogen metabolism and GPs constitute potent targets for the development of drugs and could have therapeutic value when coupled with other therapies (10, 11, 23). Consequently, the potential for structural studies of GP enzyme to provide a basis for rational drug design has long been clear, in particular using the crystallographic structure of mGP, which constituted an excellent model structure for the identification of new allosteric sites (23). So far, GP enzymes have only been studied as a potential therapeutic target for the treatment of type 2 diabetes (10, 11). Indeed, the inhibition of lGP reduces blood glucose level by lowering hepatic glucose production, thus restoring normal glycemia. Crystallographic studies allowed the identification of allosteric and pharmacological binding site used to modulate the activity of the enzyme. More recently, targeting glycogen metabolism has also been proposed for the treatment of cancers. Indeed, glycogen metabolism is reprogrammed in many cancer, leading to the accumulation of glycogen in cancer cells and tumor microenvironment, promoting cell survival (23). In particular, glycogen breakdown in cancer cells provides metabolites for the glycolysis and the production of ATP, as well as the pentose phosphate pathway which generates reducing agents and nucleotides for cell proliferation and DNA repair. As a consequence, combining the inhibition of glycogen metabolism with DNA damaging agents, ROS-generating agents and mitochondria inhibition agents might be a promising strategy for anticancer therapy (23).

In addition, glycogen performs a dual role in tissues. Indeed, on one hand, glycogen constitute a glucose supply for tissues providing metabolites for the energy metabolism and the pentose phosphate pathway (1). However, an alteration of glycogen metabolism and the subsequent accumulation of glycogen in cells is highly deleterious for the cells and lead to the development of glycogen storage diseases (24). In the brain, the inhibition of glycogen mobilization lead to impaired cognitive functions (6) and glycogen accumulation has recently been shown to be a direct cause of neurodegenerescence leading to neuronal loss and the development of Lafora disease (25). Moreover,

brain glycogen accumulation has been observed in several brain diseases including amyotrophic lateral sclerosis, Alzheimer's disease as well as in diabetes and aging (26–29). The pharmaceutical activation of brain glycogen breakdown is now proposed as a potential therapeutic target for brain diseases (30). However, so far, only GP inhibitors have been developed. Our structural data offer new perspectives for the development of specific activators. Indeed, our first structure was obtained in complex with PEG 400 used as a precipitant. Surprisingly, PEG 400 was found in the AMP binding site and induced the crystallization of the protein in an active-like conformation (12). These observations, suggest that the AMP binding site could be also targeted for the elaboration of therapeutic activators of bGP.

Finally, the structural determination of bGP and the complete image of human GP structures offers basis for the rational development of potent GP inhibitors specific from one isoenzyme that might potentially improve the efficiency of therapies and limit unwanted side effects on glycogen metabolism in other tissues.

FINAL REMARKS

The determination of the structures of the three isoforms of GP in complex with AMP and/or phosphorylated now gives a complete vision of GPs activation at the structural level. Nevertheless, the inhibition of GP enzymes by allosteric effectors, such as ATP and glucose-6-phosphate, is poorly documented. The crystal structures of mGP and bGP in complex with these ligands would thus provide fundamental new insights into the regulation of glycogen breakdown, more particularly in the brain and heart where bGP and mGP are co-expressed. We attempted several trials in order to obtain the human bGP structure in its inactive conformation. Unfortunately, bGP co-crystals with allosteric inhibitors were not obtained despite the high number of crystallization conditions tested. Nevertheless, considering the important similarities between mGP and bGP in term of structure, other structural approaches including homology modeling and molecular dynamics could provide insights on bGP and mGP inhibition by effectors. Finally, the high-resolution structures of all GP isoenzymes

will also contribute to the design of the specific drugs in order to regulate the glycogenolysis in target tissues and show promises in the elaboration of therapies for many diseases.

ACKNOWLEDGEMENTS

Work in the laboratory of FRL was supported by running grants from University Paris Diderot and CNRS. CM was supported by PhD fellowships from the French Ministry of Research (Ecole Doctorale BioSPC), We thank Dr. Salik Hussain for reading the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

CM and FRL designed the review. All authors contributed intellectually and to the writing of the manuscript.

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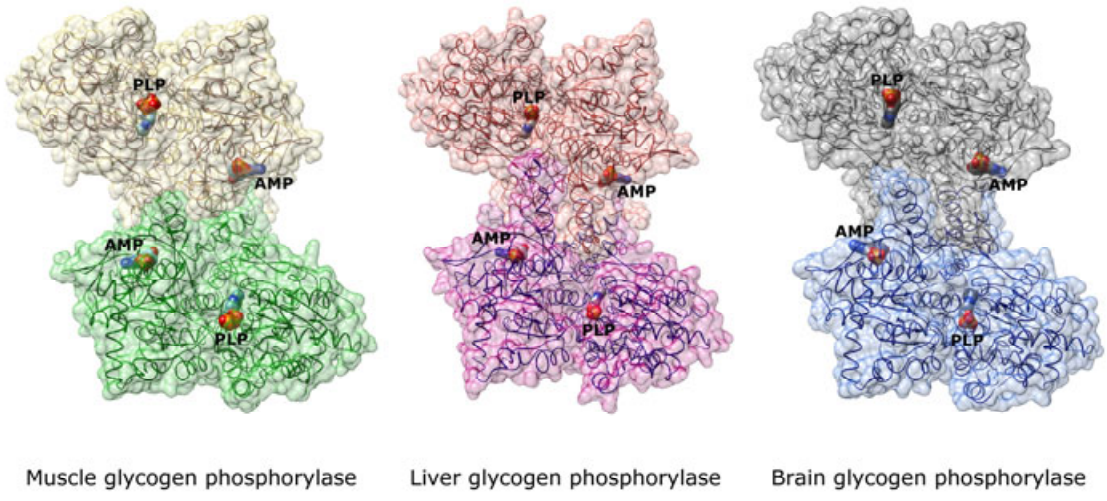

Figure 1

The three isoforms of human glycogen phosphorylase.

Surface and ribbon representation of the muscle (yellow and green), liver (orange and pink) and brain (grey and blue) isozyme of GP. The three isoenzymes form homodimers and present very similar overall structures. The PLP cofactor is present in the catalytic site of each monomer. The AMP binding site is located at the dimer interface.

(PBD ID : mGP : 1PYG, lGP :1FA9, bGP : 5IKP)

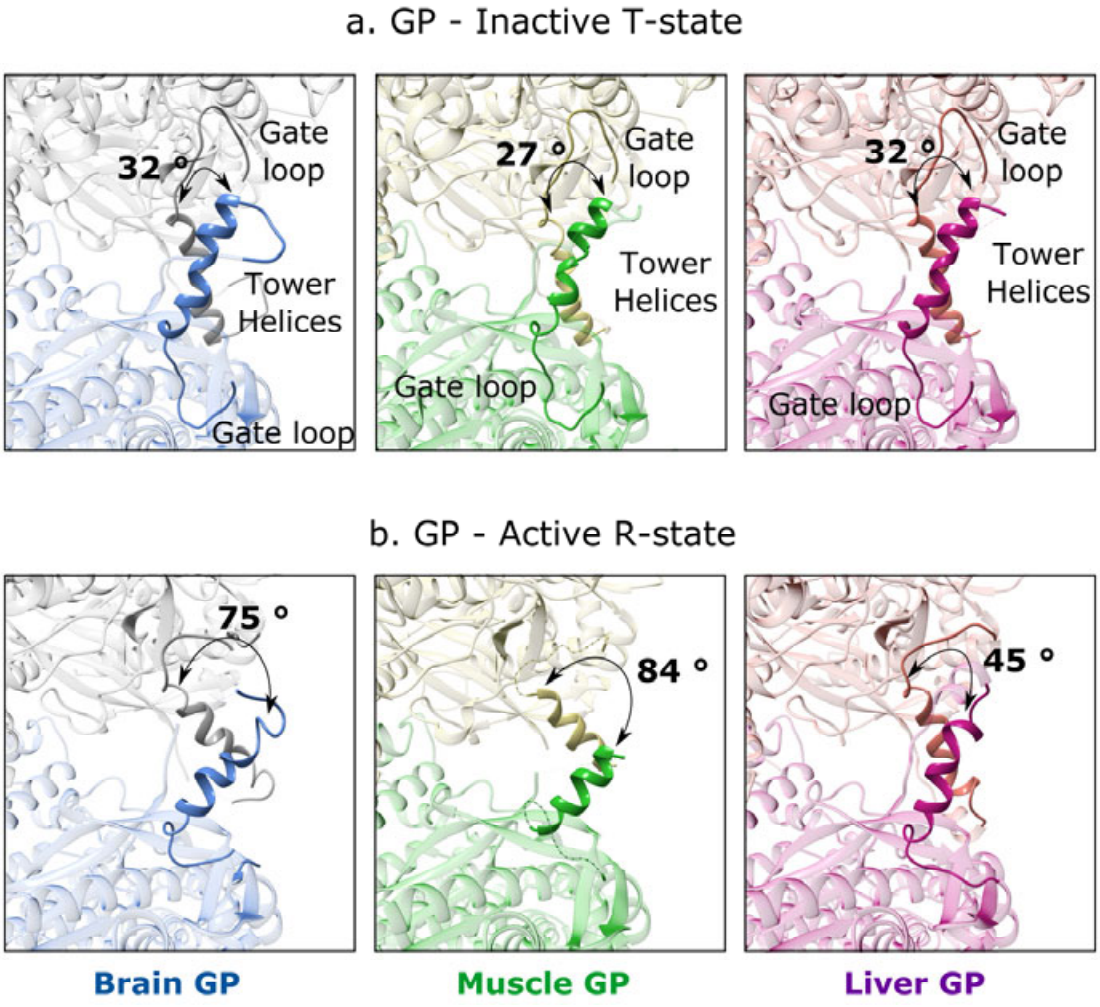

Figure 2

Rotation of the Tower helices.

Ribbon representation of the Tower helices (Helix 7 of each monomer) in the inactive (a) and active conformation of the three isoenzymes (b).

(a) The inactive state of bGP was obtained by homology modeling using inactive mGP (PDB ID : 2PYD). In the inactive state, the Tower helices display a cross over angle around 30 °.

Inactive T-state : mGP : 1GPA, lGP : 1FA0

(b) Upon activation, the Tower helices in mGP and bGP undergo a dramatic rotation of 50 ° which leads to the opening of the catalytic site, initially closed by the gate loop. On the contrary, the activation of lGP induces a compaction of the catalytic core, comprising the Gate loop and the Tower helices, which display a crossover angle of 45 °

Active R-state : bGP : 5IKP, mGP :1PYG, lGP : 1FA9

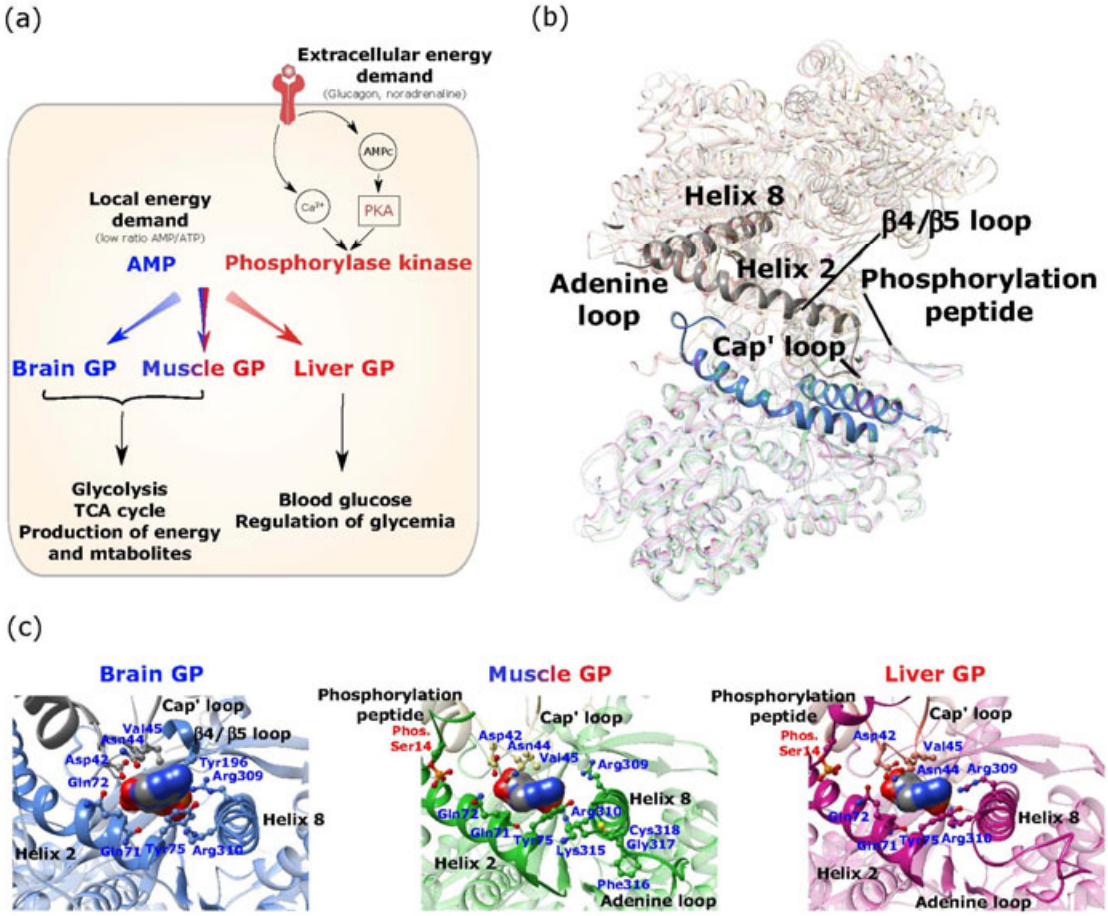

Figure 3

The functions of glycogen in humans depend on the regulation of GP enzymes.

(a) The functions of glycogen store in humans. Glycogen is mainly found in brain, muscles and liver. In brain and muscle, glycogen phosphorylase is mainly regulated by the binding allosteric effectors, thus providing energy and metabolites for brain functions and muscular contraction. In liver, the enzyme is activated phosphorylation in response to extracellular signal, including glucagon. Glycogen-derived glucose-1-phosphate is then metabolized into glucose and released in the systemic circulation for the regulation of glycemia. The isozymes that are regulated by allosteric effectors

binding (AMP, ATP) are shown in blue (brain and muscle GP). The isozymes that are regulated by phosphorylation are shown in red (mGP and lGP). mGP is both in red and blue.

(b) The structural basis of GP regulation. Structural overlay of the three active GP enzymes (bGP in grey and blue; mGP in yellow and green; lGP in pink and orange). GP enzymes organize into homodimer. Secondary structures, including the phosphorylation peptide, helices 2 and 8 as well as the cap loop and the b4/b5 loop, involved in GP activation, are highlighted. (PDB ID: bGP : 5IKP; mGP : 1GPA; lGP : 1FA9)

(c) The structural basis of bGP, mGP and lGP activation. bGP and mGP establishes several contacts with AMP in the AMP binding site (upper and middle panel), leading to the enzyme activation. AMP is marked in surface representation. Residues involved in the binding of AMP are shown. On the contrary lGP poorly associates with AMP (lower panel). In addition, the phosphorylation of Ser14 and the binding of AMP stabilizes the phosphorylation peptide in the dimer interface and stabilizes the active state in mGP and lGP, but not in bGP.

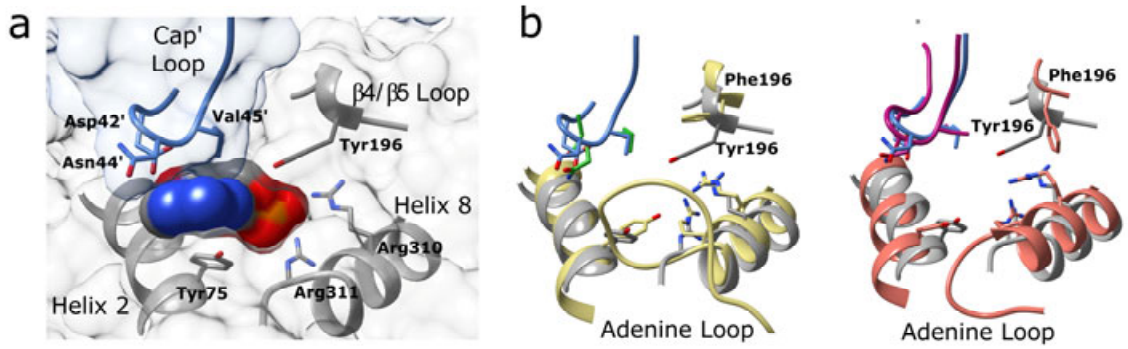

Figure 4

The AMP binding of bGP

(a) Surface and ribbon representation of the AMP binding site of bGP. AMP binding site is located at the dimer interface and comprises the Cap loop of one subunit as well as helix 2, helix 8 and β4/β5 loop from the other subunit.

(b) Structural alignment of the AMP binding site of bGP (blue and grey) and mGP (yellow and green) (left panel) and AMP binding site of bGP (blue and grey) and lGP (orange and pink) (right panel). The main regions involved in the binding of AMP are shown. The AMP located in the AMP binding site is not displayed for clarity. The substitution of Phe196 into Tyr in bGP results in the establishment of a hydrogen bond between the dimer interface of bGP and its allosteric activator. In addition, in bGP and lGP, the orientation of Tyr75 avoids the stabilization of the adenine loop.