CHAPTER 15 - GLYCOGEN METABOLISM AND GLUCONEOGENESIS

Introduction

Certain tissues, such as the brain and red blood cells, rely on glucose for fuel. Serum glucose levels must be maintained at about 5 mM.

Serum glucose is maintained by dietary sources, glycogen breakdown, and synthesis from noncarbohydrate precursors via gluconeogenesis (see Figure 1). Glucose is polymerized to glycogen (animal starch) to avoid osmotic imbalances and stored primarily in the muscle and liver. Muscle tissue maintains a store of glycogen for its own use. The liver stores glycogen stores glycogen and breaks it down to glucose for export to other tissues. Notice in the scheme below that glycogen metabolism intersects glycolysis at glucose-6-phosphate. Keep in mind that, since glycogen is stored in a muscle cell for use only in that cell, glucose-6-phosphate is never hydrolyzed to glucose in muscle cells since once glucose is formed it can leave the cell (glucose-6-phosphate cannot leave the cell).

Glycogen breakdown can be thought of as a mini-pathway that intersects glycolysis at glucose-6-phosphate. As is typically the case, there is a complementary, biosynthetic pathway that links the same endpoints. As is also typical, some of the enzymes are utilized by both catabolic and biosynthetic pathways, but at one point at the minimum, different enzymes are employed.
The importance of glycogen as a storage form of glucose is illustrated by a number of genetic diseases associated with abnormal glycogen metabolism. For example, McArdle’s disease is an inherited disease whose main symptom is muscle cramps on exertion. Although synthesized normally, glycogen breakdown is compromised resulting in inadequate supply to meet ATP needs during exertion.

**Glycogen breakdown**

Glycogen consists of glucose residues linked by "1,4 linkages with "1,6 branches every 10 residues or so. (See Figure 2 a). The breakdown of glycogen can be thought of as a mini-pathway that intersects glycolysis at glucose-6-phosphate. Glycogen is catabolized from the non-reducing end via the action of three enzymes, glycogen phosphorylase, glycogen debranching enzyme, and phosphoglucomutase.

**Glycogen phosphorylase:**

This enzyme cleaves only glucose residues attached to glycogen via alpha-1,4-linkages. Lysis of the C1-O bond provides sufficient energy to produce glucose-1-phosphate without the expenditure of ATP (Figure 15-4). This makes the storage of carbohydrate as glycogen more energy-efficient since ATP is not required to form glucose-6-phosphate (as is not the case in the case of glucose undergoing glycolysis).
Structural features:

Glycogen phosphorylase is a homodimer. The N-terminal domain contains the site of phosphorylation (ser-14), the allosteric modulator site and a glycogen binding site. The catalytic site is located at the center of the subunit.

A 30 angstrom crevice connects the glycogen binding site to the active site. In this way glycogen can bind substrate, allowing phosphorylation of several glucose residues before releasing the molecule. The crevice is too long to accommodate branch points. A branch point thus cannot be closer than 5 residues from the active site of phosphorylation.

Pyridoxal phosphate is a cofactor of phosphorylase which functions as a general acid/base
**Glycogen Debranching Enzyme**

Acts as a transferase by transferring "(1,4) linkages from a point 4 to 5 residues away from an "(1,6) branch to the non-reducing branch of another branch (Figure 15-6)

Hydrolyzes alpha(1,6) linkage at branch point (this is not a phosphorolysis). Note that free glucose is formed from glucose residues that comprise the branch point; consequently, such branch point residues, which occur every 10 residues or so, require ATP expenditure to form glucose-6-phosphate. Thus, the debranching enzyme is not as energy-efficient as glycogen phosphorylase.

Separate active sites for transferase and alpha (1,6)-glucosidase activities are located on the same enzyme, thereby increasing its efficiency.

**Phosphoglucomutase**

Catalyzes the conversion of glucose 1-phosphate to glucose 6-phosphate via the intermediate, glucose 1,6-bisphosphate. Note here the similarity to phosphoglycerate mutase, which forms the intermediate 2,3-bisphosphoglycerate. Phosphoglucomutase contains an active-site Ser to which a phosphate group is attached which adds to the substrate, whereas phosphoglycerate mutase contains an active-site His which performs the same function.

**Glycogen Synthesis**

Glycogen breakdown/synthesis occur via different pathways as do all pathways which link the same substrates. As in the breakdown/synthesis of glucose (glycolysis/gluconeogenesis), the exact reversal of the breakdown pathway would be thermodynamically unfavorable in the reverse direction. Different pathways are also utilized for control purposes, as was discussed for the case of reciprocal regulation of glycolysis/gluconeogenesis.
1 and 2 glycogen phosphorylase and debranching enzyme
3 phosphoglucomutase
4 UDP-glucose pyrophorylase
5 and 6 glycogen synthase and branching enzyme
7 inorganic pyrophosphatase

Notes:

UDP glucose is an activated form of glucose and is the second occurrence of a UDP-sugar (recall UDP-galactose in galactose catabolism). UDP is a good leaving group in this reaction.

PP_\text{i} is cleaved to provide additional driving force to this reaction which would otherwise be near equilibrium (see Figure 15-9)

UDP is formed via synthase action, and UTP is replenished from ATP via the action of nucleoside diphosphate kinase:

$$\text{UDP} + \text{ATP} \rightleftharpoons \text{UTP} + \text{ADP}$$

Glycogen synthase requires a primer, which is synthesized by another protein, glycogenin. A glucose residue is initially attached to Tyr 94 of glycogenin, which then proceeds
to extend the chain by up to 7 more residues.

**Branching enzyme**

This enzyme, distinct from the debranching enzyme, creates a branch by transferring a 7-residue from one non-reducing end of a chain to the 6-OH group of a glucose residue on either the same or another chain (see Figure 15-11).

**Control of glycogen metabolism**

Control of glycogen metabolism is more elaborate than any regulatory process we have studied thus far. We will encounter several new mechanisms here. Enzyme activation/deactivation via covalent modification occurs in addition to allosterism. The entire process is under hormonal control. We probably all know that insulin is involved in the uptake of serum glucose. This pancreatic hormone also affects glycogen metabolism. Glucagon, a polypeptide hormone stored in and secreted from the pancreas, and epinephrine (adrenaline), a catecholamine stored in and secreted from the adrenal medulla (inner part), are also involved in glycogen metabolism (see below).

The activity of glycogen phosphorylase is regulated by several mechanisms, including covalent modification, a mechanism we’re seeing for the first time. Covalent modification consists of phosphorylation of specific serine or threonine residues. This occurs at the expense of ATP and is under hormonal control.

**Allosteric Control**

In the absence of hormone, the inactive form of glycogen phosphorylase (the b form) is capable of partial activation/inactivation via the actions of the allosteric modulators AMP, glucose-6-phosphate (G6P) and ATP:
In the liver, phosphorylase-a is subject to allosteric inhibition by glucose. This is an example of feedback inhibition, since the objective of glycogen breakdown in the liver is to produce glucose for export. As we’ll see directly, glucose acts in concert with a phosphatase which removes the phosphate group subsequent to glucose binding.

Glycogen synthase is similar to glycogen phosphorylase in that it is also subject to control via both allosteric modulators and covalent modification (phosphorylation):

Notice that whereas phosphorylation initiates breakdown by activating glycogen phosphorylase, glycogen synthase is inactivated by phosphorylation, thereby ensuring that breakdown and synthesis of glycogen are not simultaneously active. The same kinase is involved in the activation of breakdown and inhibition of synthesis, thereby coordinating the reciprocal regulation of breakdown/synthesis. We have already seen how the inactive, dephosphorylated form of glycogen phosphorylase (b form) is subject to allosteric activation by energy-poor AMP, and allosteric inhibition by the energy-rich ATP and glucose 6-phosphate. We also see now that the activity of glycogen synthase can be increased by the energy-rich glucose 6-phosphate. This makes sense since an energy-poor indicator (AMP) should enhance glycogen breakdown to
provide energy, whereas energy-rich indicators should inhibit breakdown (G6P, ATP) and enhance synthesis (G6P).

Direct allosteric control of glycogen metabolism is similar to that of the committed step in glycolysis in that both this step and its reversal occur via different enzymes, as does glycogen breakdown/synthesis. Both $v_i$ and $v_r$ which determine $J$, the net flux, can be modified, allowing for more precise control. Again, control is exerted in reactions far from equilibrium because the flux through reactions near equilibrium is essentially uncontrollable.

**Covalent Modification**

As shown in the diagram above, phosphorylation of glycogen phosphorylase-b results in a conformational change producing the $a$, or active, form. Phosphorylase $a$ is more active than phosphorylase $b$ in the presence of its allosteric stimulator, AMP. The hormones that stimulate covalent modification are glucagon (and adrenaline) in the liver, and adrenaline in the muscle. Keep in mind that when the energy demands elicited by release of hormone are met, these phosphate groups must be removed. As we’ve seen before, phosphatase is the generic name of an enzyme that removes phosphate groups, and phosphatase activity must also be under hormonal control.

The enzymes involved in regulation of glycogen metabolism via covalent modification include several kinases as well as the phosphatases mentioned above. They are listed below:

**Protein kinase (cAPK).** As the name implies this enzyme phosphorylates a variety of protein substrates at the expense of ATP. The protein kinase involved in regulation of glycogen metabolism is a member of a large family of protein kinases that are important components of regulatory pathways initiated not only by hormones, but also growth factors and neurotransmitters. The protein kinase that plays a regulatory role in glycogen metabolism exists as a tetramer, with 2 catalytic and 2 regulatory subunits ($R_2C_2$). Activation of protein kinase occurs via the binding of cyclic AMP (cAMP) to the regulatory subunits, thereby causing the dissociation and subsequent activation of the C subunits:

$$R_2C_2 + 4 \text{cAMP} \rightarrow R_2(\text{cAMP})_4 + 2 \text{C (active)}$$

cAMP is produced subsequent to hormone binding to a specific receptor on the outer leaflet of the plasma membrane of a liver or muscle cell (in this case) and acts as a second, intracellular,
messenger (hormone being the primary, intercellular messenger) by allosterically activating protein kinase. The active C monomers phosphorylate specific serine or threonine residues in their target protein substrates that are part of a consensus recognition sequence, ....Arg-Arg-X-Ser or Thr-Y, where X is any small residue and Y is a large hydrophobic residue.

phosphorylase kinase As the name implies, phosphorylase kinase phosphorylates glycogen phosphorylase, thereby activating the enzyme which rapidly breaks down glycogen. The enzyme consists of 4 different types of subunits ("", $, (), and *). () is the catalytic subunit and a homolog of cAPK. The " and $ subunits are subject to phosphorylation by protein kinase, which results in activation of the () subunit by an unknown mechanism. The * subunit is calmodulin, a ubiquitous, Ca++-binding protein. In its inactive state, the () subunit of phosphorylase kinase is inactivated by a “pseudo-substrate” in the C-terminal domain of this same () subunit. The pseudo substrate is identical to the above-mentioned consensus sequence, except that the target Ser or Thr residues are replaced by a Glu residue which is incapable of being phosphorylated. Calmodulin is capable of removing this inhibitory, pseudo substrate, from the active site of the () subunit because, upon binding Ca++, the calmodulin undergoes a conformational change which exposes a hydrophobic binding site which in turn binds to, thus removing, the pseudo substrate. Activation by Ca++ is important because Ca++ also triggers muscle contraction, so simultaneous activation of phosphorylase kinase provides the fuel necessary for muscle contraction.

There is yet a third kinase, an insulin-dependent kinase which, as we’ll see, plays a role in restoring the status quo after the energy demands of the cell have been met. This kinase is a homolog of both protein kinase and phosphorylase kinase.

Phosphoprotein phosphatase 1 (PP1) dephosphorylates, thereby inactivating both glycogen phosphorylase a and phosphorylase kinase., also activates synthase by dephosphorylating it.

The action of these enzymes, the exception of the insulin-dependent protein kinase, is depicted in Figure 15-12:
Not shown in this figure is the effect of cAMP dependent protein kinase on glycogen synthase whose activity, if you’ll recall, is also sensitive to phosphorylation. In this case, however, phosphorylation inactivates glycogen synthase. The phosphorylation, thus inactivation, of glycogen synthase is catalyzed by c-AMP-dependent protein kinase.

The role of the insulin-dependent protein kinase in dephosphorylation in muscle is seen below in Figure 15-19. This kinase exerts its effect on PP1, which binds to the glycogen particle via its G subunit. Insulin will be secreted from the pancreas when the energy demands of the cell have been met. Until that point, PP1 must remain inactive. Inactivation of PP1 occurs as a result of the release of PP1 from its G subunit (and subsequent release from the glycogen particle), which is brought about by phosphorylation of one of two phosphorylation sites on the G subunit (site 2). Once released, PP1 cannot remove the phosphate groups from phosphorylase kinase, which is bound to the glycogen particle. When insulin is secreted, thus signaling an end to the rapid breakdown of glycogen, the insulin-dependent protein kinase phosphorylates another site on the G subunit (site 1), which activates PP1, resulting in removal of the phosphate groups on phosphorylase kinase. Note that protein kinase is also capable of phosphorylating site 1, but the otherwise stimulatory effects of PP1 are overridden by the phosphorylation of site 2:
In the liver things work differently. PP1 is bound to glycogen phosphorylase a but is unable to remove its phosphate groups unless levels of glucose levels are sufficiently high. Recall that in the liver, glucose is formed for export, but not in muscle. Glucose is an allosteric inhibitor of glycogen phosphorylase a and causes it to shift from the active R state to the T state. This conformational change exposes the phosphorylated ser residue for removal by PP1.

These effects are summarized in Figure 15-20, which illustrates the effects of cAMP, the various kinases, and PP1.
Note that PP1 is inhibited not only by protein kinase, the action of which releases PP1 from the glycogen particle, but also by the protein phosphoprotein phosphatase inhibitor 1. This inhibitory protein inhibits PP1 by binding to it, but it only binds to P1 when phosphorylated. The cAMP-depend protein kinase phosphorylates this protein, resulting in its binding to and inhibition of PP1. Thus, protein kinase activates the breakdown process by stimulating the enzymes responsible for breaking down glycogen, and also by inhibiting the enzymes that can remove these stimulatory phosphate groups. Note also that once activated, PP1 removes the phosphate group from glycogen synthase, thereby activating it in a reciprocal fashion.

In the absence of hormonal binding, protein kinase (cAPK), phosphorylase kinase and phosphoprotein phosphatase are dephosphorylated, thus inactive (shaded red)

Synthase is also dephosphorylated, thus active (shaded green)

PP1 is also dephosphorylated and is partially active because it remains bound to the glycogen particle and is not bound to its inhibitor, phosphoprotein phosphatase inhibitor 1, which has not yet been phosphorylated.

Upon hormonal binding, c-AMP is formed which binds to the regulatory (R) subunit of cAPK, relieving the inhibition of the C subunit.

Activated cAPK then simultaneously phosphorylates phosphorylase kinase and glycogen synthase, activating and deactivating them, respectively (note color switch).

cAPK also phosphorylates PP inhibitor 1a, which then binds to PP1, shutting it down.

The release of insulin initiates the shut down process by activating PP1, which goes about removing the phosphate groups which initiated the breakdown process.

Several other protein kinases are known to be capable of phosphorylating glycogen
Hormonal aspects

Covalent modification of enzymes involved in glycogen metabolism are under hormonal control. In the liver, the 20-residue polypeptide hormone glucagon regulates glycogen metabolism. Glucagon is synthesized, stored in and released from the pancreas in response to serum glucose levels. In muscle control is exerted by epinephrine, or adrenaline. Epinephrine, along with norepinephrine are catecholamines, derived from tyrosine.

\[
\begin{align*}
\text{Tyrosine} & \quad \rightarrow \quad \text{X = CH}_3, \text{epinephrine} \\
& \quad \rightarrow \quad \text{X = H, norepinephrine}
\end{align*}
\]

Insulin, produced, stored in and secreted from the pancreas, is also a player in glycogen metabolism, producing antagonistic effects relative to those of glucagon.

Hormones are primary, extracellular, messengers which bind to plasma membrane receptors and result in the release of cellular, or secondary, messengers such as c-AMP and Ca\(^++\). Epinephrine (and norepinephrine) bind to either \(\alpha\)-adrenergic receptors, which are linked to the adenylate cyclase system and c-AMP, or \(\beta\)-adrenergic receptors which are linked to Ca\(^++\). Binding to \(\beta\)-adrenergic receptors with the subsequent release of intracellular Ca\(^++\), reinforces the cells’ response to c-AMP, which activates glycogen breakdown:
Notes:

1. As you know, insulin is involved in glucose uptake, activating not only the receptor-mediated uptake but also cellular processes that utilize glucose, such as glycolysis, glycogen synthesis, fatty acid synthesis, etc. It has antagonistic effects with respect to epinephrine as it enhances, whereas epinephrine inhibits glycogen synthesis. Recall that the G subunit of phosphoprotein phosphatase (PP1) binds PP1 to a glycogen particle, thus activating it. This G subunit has two sites for phosphorylation (see Figure 15-19), the first of which is phosphorylated by the cAMP-dependent protein kinase (cAPK), resulting in the release of PP1 from the G unit and its consequent inactivation. This is logical as a reciprocal response to the cAMP-dependent activation of glycogen breakdown. The second site for phosphorylation is a substrate for the insulin-dependent protein kinase and results in the activation of glycogen synthesis. Thus, insulin and epinephrine can be seen to have antagonistic effects on glycogen metabolism: Insulin in its role in glucose uptake activates glycogen synthesis, whereas epinephrine in its role in activating glycogen breakdown, inhibits glycogen synthesis.

2. In this cartoon the effects of glucagon on glycogen metabolism in the liver is depicted. Glucagon is secreted in response to a need for more serum glucose (i.e., when the brain is hungry for glucose). In this event the liver will shut off its own needs for glucose and concentrate on exporting it. Not only is glycogen breakdown activated, but glycolysis is also shut down. Recall that pyruvate kinase has a regulatory role in glycolysis (catalyzes PEP to pyruvate). In the liver (but not muscle) protein kinase phosphorylates, among its various substrates, pyruvate kinase,
thus inhibiting it. This demonstrates how phosphorylation via the cAMP-dependent protein kinase cascade results in the export of glucose from the liver by not only enhancing glycogen breakdown but also (reciprocally) inhibiting glycolysis via its effect on pyruvate kinase.

3. In order to export glucose from the cell, glucose 6-phosphate must be hydrolyzed to glucose via the action of glucose 6-phosphatase. This enzyme is located almost exclusively in the liver because of the liver’s role in exporting glucose. Muscle cells, for example, contain no glucose 6-phosphatase because their glucose is targeted for internal use.

**Gluconeogenesis**

Sources of glucose are dietary, via glycogen breakdown and from noncarbohydrate precursors by gluconeogenesis. These include lactate, pyruvate, citric acid intermediates and most amino acids (except leucine and lysine). Amino acids that can be converted to glucose via gluconeogenesis are termed glucogenic. Those that cannot are termed ketogenic. All precursors to glucose are funneled into gluconeogenesis via oxaloacetate. Ketogenic amino acids are those that are ultimately converted to acetyl CoA, not oxaloacetate. As we have seen, the aerobic fate of pyruvate is to be converted into acetyl CoA in the mitochondria. However, this reaction is not reversible, hence acetyl CoA is destined to enter the citric acid and thus cannot, as we will see, ultimately be converted into glucose. Interestingly, fats are broken down into acetyl CoA as well and thus cannot be converted into glucose. Keep such things in mind as you construct your metabolic road map and connect various pathways.

(Figure 15-22) Employing all the enzymes of glycolysis in reverse would accomplish the conversion of pyruvate to glucose. Although gluconeogenesis does employ several glycolytic enzymes in the reverse direction, not all the reactions of gluconeogenesis are the reverse of glycolytic reactions. There are at least two important reasons for this. Since glycolysis is exergonic, its exact reversal would be endergonic, hence energetically unfavorable. Secondly, if glycolysis and gluconeogenesis occurred via the same enzymes operating in different directions, regulation of both would be problematic because the net flux would then be primarily controlled by mass action effects. The reactions in Figure 22 connected by sets of straight arrows in depicting different directions (W) are catalyzed by the same enzymes in glycolysis and gluconeogenesis. Only those reactions connected by pairs of curved arrows occur via different enzymes.
catalyzed by a kinase in glycolysis at the expense of ATP hydrolysis. By simply removing the phosphate groups in gluconeogenesis via the action of the appropriate phosphatase, the necessity of having to synthesize ATP in the reverse direction, a highly endergonic process, is avoided. These two reactions, then, solve the thermodynamic problem.

2. Regulatory aspects also come into play here. Recall that both of these reactions have regulatory roles in glycolysis, and the second is the key regulatory step in glycolysis. Both negative (ATP) and positive (fructose 2,6-bisphosphate = F2,6P) modulators affect phosphofructokinase (PFK). These modulators affect fructose bisphosphate, but in different, complementary fashion. Thus F2,6P, although a potent allosteric activator of phosphofructokinase, is a potent allosteric inhibitor of fructose bisphosphatase (FBPase). This important modulator is produced (from fructose 6-phosphate) and degraded by different versions of PFK and PBPase (PFK-2 and PBPase-2). Both enzyme activities are located on the same - 100 kDa homodimeric protein. This bifunctional, tandem enzyme (TE) is subject to both allosteric modulators and regulation by covalent modification. Allosteric modulators include fructose 6-phosphate which activates the kinase and inhibits the phosphatase activities, thus activating glycolysis (and inhibiting gluconeogenesis), which you would expect in the presence of ample
fructose 6-phosphate levels). The tandem enzyme has a susceptible serine OH group which can be phosphorylated by the cAMP-dependent protein kinase (cAPK):

To summarize the effects of glucagon on various pathways in the liver via the cAMP cascade:

Glycogen breakdown is stimulated by conversion of glycogen phosphorylase b to phosphorylase a (active form)

Glycogen synthesis is inhibited by inactivation of glycogen synthase

Glycolysis is inhibited and gluconeogenesis reciprocally stimulated by
Phosphorylation of the tandem enzyme that forms fructose 2,6-bisphosphate

Glycolysis is inhibited at another regulatory point by phosphorylating pyruvate kinase.

Note that in heart muscle the effects brought about by the cAMP cascade via epinephrine are different than they are in liver, as one would expect since the metabolic goals in liver and muscle are different. In heart muscle phosphorylation of the tandem enzyme activates PFKase-2-activates rather than inhibits it, thus activating glycolysis. In this case glucose is broken down in the heart to meet energy demands, whereas in the liver glucose is exported.

The reversal of the last reaction in glycolysis, PEP to pyruvate, is catalyzed by two different enzymes in gluconeogenesis. The pyruvate to oxaloacetate conversion is catalyzed by pyruvate carboxylase at the expense of ATP hydrolysis, and requires biotin:
Note that this mechanism differs from that depicted in Figure 15-25 and is more plausible.

The energy cost per glucose from pyruvate via gluconeogenesis is seen to be 6, counting the 2 ATP that must be formed via a reversal of the substrate level phosphorylation in glycolysis.

Miscellaneous comments on regulation of gluconeogenesis:

Final comments on regulation of gluconeogenesis:

Acetyl CoA, formed from pyruvate during its anaerobic degradation in the mitochondria will be seen to enter the citric acid cycle, producing the remainder of the 36 to 38 ATP per glucose while it is converted to CO₂. Acetyl CoA, as an energy-rich indicator, activates pyruvate carboxylase, thus gluconeogenesis. Pyruvate kinase, a glycolytic enzyme, is inhibited by alanine, a major gluconeogenic precursor. Alanine is easily converted to pyruvate via transamination:
Note that oxaloacetate, a citric acid cycle intermediate, cannot leave the mitochondria. Thus pyruvate must enter the mitochondria where it is converted to oxaloacetate by pyruvate carboxylase. Then it is converted to either malate or aspartate, either of which are exported via the malate shuttle (see Figure 15-27). When oxaloacetate leaves as malate, note that reducing power in the form of NADH will also be exported, which is likely desirable since gluconeogenesis requires it in the reduction of 1,3-bisphosphoglycerate to 3-glyceraldehyde. A simplified way of viewing this is:

Problems: 5, 6, 7, 8, 10