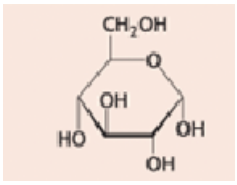
This guide is meant to walk you through the material for Exam 4 in Biochemistry. This will cover glycolysis, gluconeogenesis, the PDH Complex, the TCA cycle, and all of their regulators. This is meant to be more of a first walk through, and should set you up for Jordan's slides once you feel more confident. Good luck, and make sure to review your own notes as well!

Glycolosis

So to start, I am going to walk you through glycolysis and try to help it make a bit more intuitive sense. It is always good to try and understand *why* as it almost always aids in memorization (and if you end up getting stuck on the exam, you can try and reason your way through)!

1. **Glucose** (Our starting material!)

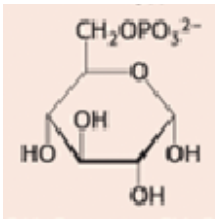


ENZYME: Hexokinase (irreversible)
Naming explanation:

- Hexokinase: hex means six
- Hexokinase: a kinase is something that transfers a phosphate group from high donor molecules.
- Hexokinase: adds a phosphate group to the 6th carbon from ATP

Note: The step of adding a phosphate group traps the glucose in the cell

2. **Glucose-6-phosphate**

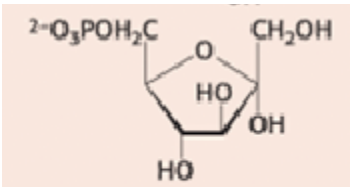


You can see the phosphate group is now on carbon 6!

ENZYME: phosphoglucose isomerase
Naming explanation:

- Phosphoglucose: phosphate+glucose
- Isomerase: Makes an isomer (changes the arrangement of the molecule)
- Phosphoglucose isomerase: makes an isomer from glucose with a phosphate on it.

3. **Fructose-6-phosphate**



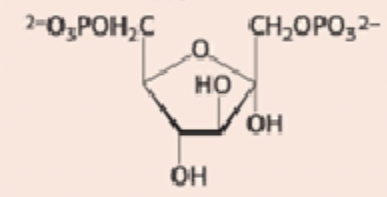
It is now a fructose sugar molecule (5 member ring instead of 6) but still has the phosphate on carbon 6
Remember: we did not lose any atoms, they were simply rearranged.

ENZYME: Phosphofructokinase (irreversible)
Naming Explanation:

- Phosphofructo: phosphate+fructose
- Kinase: again, a kinase is something that transfers a phosphate group from a high donor molecule.
- Phosphofructokinase: adds a phosphate group from ATP onto fructose-6-phosphate.

NOTE: RATE LIMITING STEP

4. **Fructose-1,6-bisphosphate**

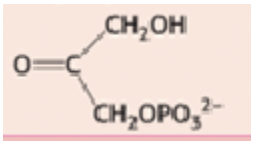


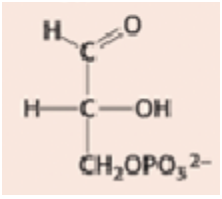
Fructose with 2 phosphate groups in it! One on carbon 6 and now one on carbon 1.

ENZYME: Aldolase
Naming explanation:

- Aldolase is an enzyme that makes aldose
- GAP (one of the next molecules it makes) is an aldose. The other molecule it makes, DHAP, is a ketose, which will later have to be rearranged to become GAP as well.

5. **Dihydroxyacetone (DHAP) + Glyceraldehyde 3-phosphate (GAP)**





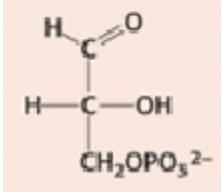
ENZYME: Triose phosphate isomerase

- Triose: three carbon sugar
- Phosphate: has a phosphate group
- Isomerase: rearrange the molecule to make an isomer
- Triose phosphate isomerase: Modify a 3 carbon sugar with a phosphate group into an isomer (GAP).

*Note: look at the two structures and think about how the isomerase will have to rearrange DHAP to make GAP.

~This is the end of the Energy Input Phase, now we are entering the Energy Output Phase~

6. **GAP (x2)**



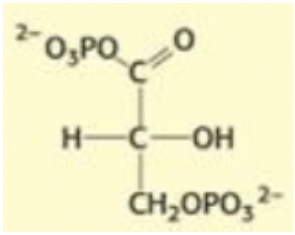
ENZYME: Glyceraldehyde 3-phosphate dehydrogenase

- Glyceraldehyde: GAP

- 3-phosphate: phosphate on carbon 3
- Dehydrogenase: loses a hydrogen
- Glyceraldehyde 3-phosphate dehydrogenase: This adds a phosphate on carbon 3 of GAP by losing a hydrogen to NAD+.

Note: NAD+ is now converted to NADH.

7. **1,3-Biphosphoglycerate (X2)**



The previous enzyme added a phosphate group, which is now why the name is 1,3- *Bi*phosphoglycerate

ENZYME: phosphoglycerate kinase

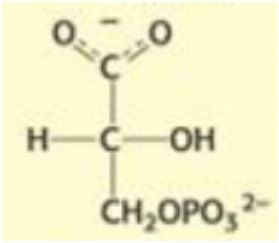
Naming explanation:

- Phospho- Phosphate
- Glycerate- Glycerol! (Our 3 carbon sugar)
- Kinase- adds a phosphate group from a high energy donor

*Note: This kinase here makes it seem like we are going to need an input of ATP to make this. However, the 1,3-Biphosphoglycerate is now the donor, and adds a phosphate group to ADP.

<u>Yield: 1 ATP per GAP molecule → 2 ATP molecules in total are made.</u>

8. **3-phosphoglycerate (X2)**



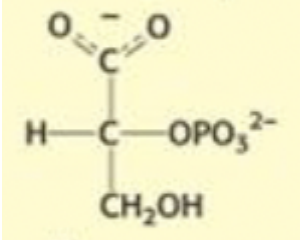
As you can see, one of the phosphate groups has been removed!

ENZYME: Phosphoglycerate Mutase

Naming Explanation:

- Phospho- Phosphate
- Glycerate- Glycerol (Our 3 carbon sugar) ((Notice this does not change much since the backbone stayed the same))
- Mutase: So these are a group of isomerases that specifically tend to only move phosphate groups. (Also, I like to think about the fact that they sound like "mutate" so they are rearranging the molecule)

9. **2-phosphoglycerate (X2)**



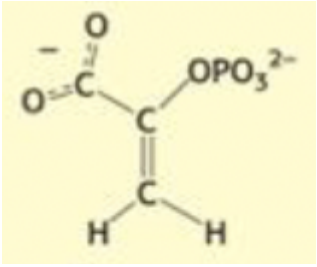
If you notice, all that changed here was the position of the phosphate. It is now on carbon 2 instead of carbon 3.

Why?

This structure is important to then lead to our high energy intermediate (PEP) and catalyze the production of another ATP molecule.

ENZYME: Enolase
Naming Explanation:
Enol: Have a C=C double bond! → Enolase, creates that double bond!

10. Phosphoenolpyruvate (X2) (PEP)



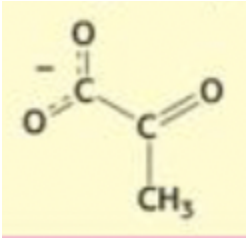
From the last enzyme, you can see the term "enol" within the new molecule!
So now the molecule has a phosphate group, an enol, and a pyruvate group.

ENZYME: Pyruvate Kinase (irreversible)
Naming Explanation:

- Pyruvate→ pretty simple..
- Kinase: moves a phosphate group from a high energy donor.

Note: Again, our high energy donor is NOT ATP but is phosphoenolpyruvate
Generates <u>ATP</u> from ADP

11. Pyruvate (X2) *Final Product!*



<u>To sum up the cycle</u>:

1. Energy investment phase:
    a. 2 ATP
        i. One from hexokinase (to form Glucose-6-phosphate)
        ii. One from phosphofructokinase (to form Fructose-1,6-bisphosphate)
2. Energy payoff phase:
    a. 4 ATP
        i. 2 ATP from Phosphoglycerate kinase (to generate 3-phosphoglycerate X2)
        ii. 2 ATP from pyruvate kinase (to form pyruvate X2)
    b. 2 NADH
        i. Glyceraldehyde 3-phosphate dehydrogenate (to form 1,3-Biphosphoglycerate)

"Net Yield" : This refers to the total amount of energy producing molecules we gained while subtracting the ones we used in the reaction.
→ We have a net gain of 2 ATP (because we used two) and 2 NADH (because we did not use any).

Allosteric Regulation in Muscle
(Glycolosis)
Regulation of Glycolysis in Skeletal Muscle, Regulation of Glycolysis in Skeletal Muscle (Part II) I would recommend watching this video to go over this! He does an amazing job of explaining all of the regulatory steps.

1. Phosphofructokinase
    a. Inhibited by ATP
    b. Activated by AMP
    c. Inhibited by low pH (high H+)

~Phosphofructokinase is the enzyme that catalyzes the phosphorylation of Fructose-6-phosphate to make Fructose-1,6-bisphosphate.
~Additionally, it is the key step in glycolysis. Once this enzyme adds the phosphate group, it is committed to becoming pyruvate. Otherwise, it can follow another pathway and become glycogen (since glucose also works to build fatty acids).
What is AMP?

- When there are low levels of ATP, there is an enzyme "adenylate kinase" which will take two ADP molecules and convert them to ATP and AMP by moving a phosphate.
    - ADP + ADP→ ATP + AMP
    - (ATP: Adenosine TRIphosphate; ADP Adenosine DIphosphate; AMP: Adenosine MONOphosphate.)

So, why would phosphofructokinase be inhibited by ATP but activated by AMP?

- Each of these molecules are indicative of an energy "state" in the body.
    - "The Energy Charge Ratio" → ATP/AMP; this tells us how much ATP, or energy, our cells have.
    - Conceptually, if ATP is really high, we have a lot of energy (aka, a high energy charge ratio), if AMP is really high, we have low amounts of energy (aka, a low energy charge ratio).
        - AMP is a low energy state as it tells us that the cell has to convert ADP to AMP and ATP to get energy, letting us know that we may be depleting our resources and need more ATP.
- With that in mind, we can say that when we have high ATP, we do not need more energy. This also means that we are not using much energy, so this is indicative of the *resting* state.
- On the other hand, when we have high AMP (or low ATP), we are in need of more energy, which would tell us we are using a lot and are in the *active* state.

What happens?

- At high levels of AMP, AMP will bind to phosphofructokinase and activate it. It binds with a HIGHER affinity than ATP!

- At high levels of ATP, ATP will bind to phosphofructokinase and inhibit it.

Why does H+ (low pH) inhibit phosphofructokinase?

In the absence of oxygen, cells undergo lactic acid fermentation using the products of glycolysis. It results in the build up of lactic acid, which increases acidity (Increasing H+ concentration, and lowering pH). In order to prevent tissue damage from the acidic environment, phosphofructokinase is inhibited, and does not allow the reaction to proceed.

2. Hexokinase
    a. Inhibited by Glucose-6-phosphate.

If you remember, hexokinase converts glucose into glucose-6-phosphate by adding a phosphate group.

- Glucose-6-phosphate and fructose-6-phosphate stay in equilibrium in the cell→ due to this, if phosphofructokinase is inhibited, fructose-6-phosphate will be increased, and therefore so will glucose-6-phosphate. → this inhibits hexokinase from converting glucose at all.

Note* This inhibition has a low Km, so only a slight excess of glucose-6-phosphate will cause inhibition.

3. Pyruvate Kinase
    a. Inhibited by ATP

Pyruvate kinase generates the final ATP from PEP.

- If you have enough ATP, do not need to convert it!

Feedback Loop:

Fructose-1,6-biphosphate activates pyruvate kinase.

Allosteric Regulation of the Liver

Regulation of Glycolysis in Liver Cells (Until 12:30)

1. Phosphofructokinase
    a. Inhibited by: citrate
    b. Inhibited by: ATP

- The same thing happens with ATP
- Citrate now inhibits PFK (I am starting to get tired of writing it out so much…) . This is because citrate is the first product of the TCA cycle in the mitochondria, so if it is in excess, the liver knows that it does not need to produce any more as there will be enough ATP made, and it can store glucose as glycogen.
    - What citrate does is increase the ability of ATP to bind to and inhibit PFK.

2. Pyruvate Kinase
    a. ATP inhibition
    b. Alanine inhibition

Again, ATP works the same.

- However, another pathway that pyruvate can follow is to eventually form alanine. When there is an increase of alanine in the cell, pyruvate kinase knows that it does not need to keep producing pyruvate since it is not needed, and will be inhibited.

While we are on the topic of pyruvate kinase, there is something strange it does in liver cells.
→ It has an extra layer of inhibition built in that is not present in muscle cells.

- Phosphorylation!
- When it is phosphorylated, it is less active. When it is unphosphorylated, it is more active.
- At HIGH blood glucose levels, it is dephosphorylated
- At LOW blood glucose levels, it is phosphorylated.

Why?

Our liver is meant to regulate the amount of glucose in the blood. It does not want there to be too much or too little. Therefore, when we are in a fasted state, we need to figure out a way to keep glucose levels relatively constant. To do this, a kinase comes along and phosphorylates pyruvate kinase to lower its activation. This allows for the accumulation of PEP, which allows for the process of glucogenesis to reestablish the levels of glucose in the blood!

Fermentation

For glycolysis to keep running, we need NAD+ (remember… to take the hydrogen in step 6)
Under aerobic circumstances where the pyruvate would eventually enter the TCA cycle and the ETC, these hydrogens would be removed for energy and replenished.
However, under anaerobic conditions, this does not occur.
→ Lactic acid fermentation! (In animals)

- Organisms can regeneration NAD+ by reducing pyruvate into lactate + NAD+
- This is done by the enzyme <u>lactate dehydrogenase</u>
- Occurs in red blood cells, and with intense exercise.

In yeast, it does Ethanol fermentation
This has two steps:

1. Pyruvate → Acetylaldehyde + CO2
    a. By <u>pyruvate decarboxylase</u>
2. Acetylaldehyde + NADH → NAD+ + Ethanol
    a. <u>Alcohol dehydrogenase</u>

notice a pattern in the naming of enzymes?

Gluconeogenesis
Making glucose!

1. **Pyruvate (or lactate/amino acids) X2 + ATP + CO2→ Oxaloacetate + ADP**

We are starting with the ending material for glycolysis!

ENZYME: Pyruvate carboxylase *NEW*
Naming:
- Pyruvate…
- Carboxylase: an enzyme that adds carbon dioxide.

→ Input: ATP + CO2
→ Output: ADP

2. **Oxaloacetate (or amino acid) X2 + GTP → Phosphoenolpyruvate + GDP + CO2**

This is an intermediate that is not needed in glycolysis
(Reminder: this gets transported out of the mitochondria to the cytosol)

ENZYME: Phosphoenolpyruvate carboxykinase
Naming:
- Phospho: Phosphate
- Enol: double carbon bond C=C
- Pyruvate: precursor to pyruvate
- Carboxy: carbon dioxide (LOSE CO2 molecule)
- Kinase: transport a phosphate group from a high energy donor (GTP→ GDP)

*Input: GTP,
Output: GDP, CO2

3. **Phosphoenolpyruvate X2 → 2-phosphoglycerate**

This is our first intermediate from glycolysis
(from here, the reaction is the same until step 8)

ENZYME: Enolase
- REMOVES double bond (does the reverse reaction)

4. **2-phosphoglycerate X2 → 3-phosphoglycerate**

ENZYME: Phosphoglycerate Mutase

Naming:

- Phospho: phosphate
- -Glycerate: glycerol
- Mutase: isomerase that just moves the phosphate group

Remember: reverse reaction!

5. **3-phosphoglycerate X2 → 1,3-Biphosphoglycerate**

ENZYME: Phosphoglycerate kinase
Naming:
Phosphoglycerate: glycerol with phosphate
Kinase: move phosphate group from high energy donor.
→ Input ATP
→ Output: ADP
Note: remember, we received an ATP from glycolysis, so in this step, we will need to spend an ATP for the reverse reaction.

6. **1-3-Biphosphateglycerate X2 → GAP**

ENZYME: GAP-3-Phosphate dehydrogenase
Naming:
GAP: Our next intermediate!
3-Phosphate: affects the phosphate on carbon 3
Dehydrogenase: reverse reaction→ uses a hydrogen
→ Input: NADH
→ Output: NAD+

7. **GAP or Glycerol (**if glycerol, needs to be modified to GAP by enzyme triose phosphate isomerase→ much like DHAP) **X2 → Fructose-1,6-Bisphosphate**

ENZYME: Aldolase

8. **Fructose-1,6-Bisphosphate → Fructose-6-phosphate**

NEW STEP
RATE LIMITING
ENZYME: Fructose-1,6-bisphosphatase
Naming:
This name makes a bit of intuitive sense, as it removes the phosphate (phosphatase is a molecule that cleaves a phosphate) and adds a hydrogen from H20 to this molecule.

9. **Fructose-6-phosphate → Glucose-6-phosphate**

ENZYME: Phosphoglucose isomerase

10. **Glucose-6-phosphate**

NEW STEP

ENZYME: Glucose-6-phosphatase
Same thing as before! Phosphatase removes a phosphate group! Adds hydrogen instead from water.
Note: Located in the ER in liver cells.

11. **Glucose!** (Final product)

To sum it up:
-We used 4 molecules of ATP and 2 molecules of GTP per glucose (2 ATP and 1 GTP per pyruvate)
- Be careful with this, as you may be asked how much "ATP" was used, which excludes GTP, and how many "ATP equivalents" were used, which would include GTP.
-2 NADH molecules

Regulators of Gluconeogenesis:

1. Pyruvate carboxylase
    a. Acetyl-CoA→ allosteric activator
        i. Why? →
    b. ADP inhibits
2. Phosphoenolpyruvate carboxykinase
    a. ADP inhibition
3. Fructose-1,6-biphosphatase
    a. Citrate activates
    b. AMP inhibits
    c. F-2,6-BP inhibits

The rest are basically the opposite of what happens in glycolysis. → They experience *reciprocal regulation*. This is because when one is active, it is in the cells best interest to have the other be inactive.

Hormonal Regulation:

- So we know the liver's role is to maintain blood-glucose levels. It does this by using fructose-2,6-Bisphosphate.
- Reminder: This is NOT the same thing as Fructose-1,6-Bisphosphate, and actually is not involved in glycolysis or gluconeogenesis at all, besides its ability to activate or inhibit enzymes.

Which enzymes!?
It STIMULATES phosphofructokinase but INHIBITS fructose-1,6-bisphosphatase

Concept check:
What does this tell you about when the liver would want to make fructose-2,6-bisphosphate?

(A. It would want to make fructose-2,6-bisphosphate when it wants to undergo glycolysis and NOT undergo gluconeogenesis. → phosphofructokinase is stimulated, which will catalyze the step towards generating pyruvate in glycolysis, and will inhibit fructose-1,6-bisphosphate from generating glucose) → Insulin will activate F-2,6-BP synthesis

What generates F-2,6-BP?
PFK-2
Again, not the same thing as PFK (phosphofructokinase). This enzyme has 2 functional domains that make and break down F-2,6-BP.

*Note: Think of the liver as being "self-less" → it stops producing sugar when glucose levels are low.

State	Hormone signal	PFK-2 (Liver isoform)	[F-2,6-BP]	Consequence for PFK-1	Consequence for FBPase-1	Net pathway favored
Fed	↑ Insulin	Kinase active (dephosphorylated)	↑ High	Strong activation	Strong inhibition	**Glycolysis**
Fasted / stress	↑ Glucagon or ↑ Epinephrine	Phosphatase active (phosphorylated)	↓ Low	Low activity; Inhibited by ATP	Active	**Gluconeogenesis**

Cori Cycle: this cycle illustrates how the liver and muscle cells work together.
→ Lactate produced by the muscles during contraction goes into the blood, the liver removes them and converts it into glucose, which is again released into the blood.

PDH Complex
The PDH complex is what generates Acetyl-CoA from pyruvate to begin the TCA cycle.
Irreversible
The PDH had three complexes:

E1: Pyruvate dehydrogenase
This is named after the complex itself!
Cofactor: TPP

- This is used in the first step, where it catalyzed the activity of pyruvate interacting with TPP (Thiamine pyrophosphate) to form Hydroyethyl TPP

E2: Dihydrolipoyl transacetylase
Cofactor: Lipoic Acid (Also uses CoA)

- The lipoic acid extends out from the complex and moves each intermediate to its respective complex!

E3: Dihydrolipoyl dehydrogenase
Cofactor: FAD

So what happens? Pyruvate Dehydrogenase Complex
Unfortunately, I do not have a good way to type this into the document, so please watch the video!

Regulation: Regulation of Pyruvate Decarboxylation
Covalent modification→ Phosphorylation!
When it is phosphorylated→ INACTIVE
When dephosphorylated → Active

PDH Kinase phosphorylates the E1 complex
→ Activators: ATP, Acetyl CoA, NADH (This tells us there is a build up in the cell)

PDH Phosphatase dephosphorylates the E1 complex
→ Activators: Ca2+, ADP (Tell us we NEED to make energy).

Additionally, E2 is *inhibited* by acetyl-CoA and E3 is inhibited by NADH (which makes sense due to what they each make…)

TCA Cycle:

Phase 1: Acetyl Entry

- This phase gets the molecule ready for the following steps.

1. **Acetyl CoA + Oxaloacetate → Citrate + CoA-SH**

ENZYME: Citrate Synthase

- This one is early to remember, as it literally synthesizes citrate!

Note: This step is irreversible*
→ Inhibited by: ATP, NADH, and Citrate

2. **Citrate → Isocitrate**

ENZYME: Aconitase

- Now this name is not intuitive, so it may take some strange memorization tricks. For me, I know this reaction is a dehydration and rehydration reaction, so I think of aconitase as "I can not trace" meaning that it moves things around but nothing is missing!

Phase 2: Oxidative Phase

- This phase produces NADH and CO2 from both reactions

3. **Isocitrate → alpha-Ketogluterate + CO2 + NADH**

ENZYME: Isocitrate dehydrogenase **RATE LIMITING**

- Inhibited by→ ATP, NADH
- Activated by → ADP and Ca2+ (Why? → Ca2+ is around after muscle contraction)

4. **alpha-Ketogluterate → Succinyl-CoA + CO2 + NADH**

ENZYME: alpha-Ketogluterate dehydrogenase COMPLEX (this is the same structure at PDH and functions the same)

- Inhibited by: Succinyl-CoA, ATP, NADH

Phase 3: Energy Capture

- In this phase, GTP is generated.

5. **Succinyl-CoA → Succinate + CoA + GTP**

ENZYME: Succinyl-CoA Synthetase

- This one kind of stinks since it does not make succinyl CoA…

- This gets its energy from the thioester bond.

Phase 4: Regeneration Phase

- In this phase, oxaloacetate is regenerated

6. **Succinate → Fumarate + FADH2**

ENZYME: Succinate dehydrogenase (Complex II) → had FAD on it

7. **H2O + Fumarate → L-Malate** (yes, it is stereospecific)

ENZYME: Fumarase

8. **Malate → Oxaloacetate + NADH**

ENZYME: malate dehydrogenase

- This reaction is unfavorable, BUT the pull for oxaloacetate to form citrate is so strong that it overcomes it.

To sum it all up:

2 GTP were generated → 2 ATP
6 NADH will indirectly produce → 15 ATP
2 FADH2 will indirectly produce → 3 ATP

=20 ATP produced (only 2 directly…)