

I am confused about the PFK-2 enzyme. On slide 18 it shows the enzymatic activity (affinity for F6P) of PFK-2 is reduced when no F26BP is present. Why? If PFK-2 converts F6P to F26BP, shouldn't its activity increase when there is no F26BP in the environment?

We need to separate the functions of the two PFK enzymes:

PFK-1 is the glycolytic enzyme that we primarily want to regulate.

PFK-2 is the enzyme that produces fructose-2,6-bisphosphate, an activator of PFK-1

In the presence of F-2,6-BP, PFK-1 is more active, as shown in that figure. Varying the concentration of F-6-P and measuring the rate, is the method we use to generate a standard Michaelis-Menten plot for an enzyme. Notice the activity of PFK-1 is higher in the presence of F-2,6-BP at any substrate concentration.

Of course, you are right, F-6-P is also a substrate for PFK-2, but that is not what this figure shows, and there is no PFK-2 in this experiment – only its product, F-2,6-BP.

What exactly does phosphopentose isomerase do?

It converts aldophosphopentoses to ketophosphopentoses and vice versa.

We saw the interconversion of ribulose-5-phosphate (a ketopentose phosphate) with ribose-5-phosphate (an aldopentose phosphate).

The chemistry: the enzyme converts the carbon-carbon single bond between carbons 1 and 2 into a double bond through a rearrangement. The intermediate is an ene-diol that can then react either way – to form a ketone, or an aldehyde (ketose or an aldose). This is a common mechanism that we also see in phosphohexose isomerase.

Could you go over how Fructose - 2,6 - Bisphosphate interacts with the regulation of PFK? Is this product related to glycolysis because F-6-P can also be made into this? Can you also go over the part concerning the "single polypeptide with opposing activities (PFK-2)" and how phosphorylation of the enzyme works.

The activity of PFK-2 is reciprocally regulated with the enzyme that has its opposite activity – removing the phosphate on carbon 1 of fructose – and these two enzymes are on the same polypeptide. This is common because with a single modification, you can turn one enzyme on while turning the other off and vice versa.

F-2,6-BP interacts allosterically with PFK-1 to

Do we have to know the supplemental slides for the exam?

No, but I would certainly look them over. They are for your own information and hopefully they can supplement some of the topics we cover in lecture.

Are the malate and aspartate transporters in gluconeogenesis the same as the malate and aspartate transporters used re transport of nadh into the matrix? I note that the malate transport for gluconeogenesis is in the opposite direction as the one for nadh transport.

Yes this is the malate/ α -ketoglutarate transporter.

If they *are* the same transporters, does that mean that the cells that use the malate aspartate shuttle can do gluconeogenesis using pyruvate, but that the cells that use the glycerol 3 phosphate shuttle cannot do gluconeogenesis via pyruvate?

The tissues that primarily use the glycerol-3-phosphate shuttle - the brain and skeletal muscle – do not carry out much gluconeogenesis.

Pyruvate gets through the outer membrane of the mito by facilitated diffusion, and through the inner membrane by active transport. And even though active transport is required no ATP are used up. Am I on the right track with this?

The transport is energy dependent, but the energy likely comes from the proton gradient.

When glucose levels are low and FBPase is activated (which means more F26P is used up) how does this activate PFK-1? I thought the more F26P, the better for (more active) PFK-1 Am I missing something?

Active phosphatase doesn't activate PFK-1, it lowers its activity. In a low glucose state you don't want PFK-1 to be active because there is no glucose to metabolize. You want F-2,6-BP to be low.

Will you be posting the exam from last year?

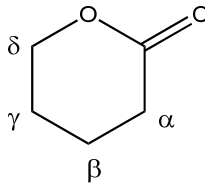
No, but I will post some sample questions.

Hi professor, can you give me some suggestions on how to best prepare for the upcoming midterm? Do I need to know how to draw every structure? And for the pathways do I need to draw and name every enzyme and structure for example glycolysis or TCA. For example being able to draw the complete TCA cycle or the entry of galactose into glycolysis? Any tips on what to study so I can be best prepared for the 1st midterm will be much appreciated thank you.

Okay, first you should know all structures and pathways - this is very important. Once you know the pathways, then you can really start to understand the pathways. I want you to learn how metabolism works and how molecules are interconverted to meet the needs of the organism. Try to visualize each pathway as a small part of a larger machine and how everything works together.

What does the δ (delta) refer to in 6-phosphoglucono- δ -lactone?

It is the notation given to six member lactone rings. Five membered lactone rings are given the designation γ -lactones and four membered lactone rings are known as β -lactones. These arise from the notation of carbons after the carbonyl as α , β , γ , δ ,.....et cetera. See below.



The enzyme that converts glyceraldehyde into glyceraldehyde-3-Phosphate is called Glyceraldehyde Kinase in Voet & Voet but its called Triose Kinase in the notes. Is there any difference?

Nope, they are the same thing. Enzyme names vary from textbook to text book based on the author(s) preferences. Some enzymes have many aliases.

Gluconeogenesis takes place in the liver right?

Primarily in the liver and kidneys. Very little gluconeogenesis occurs in the brain or muscles.

Is glucose-6-phosphatase located in the endoplasmic reticulum lumen? Does this mean that the reaction that yields glucose takes place there too?

It is a membrane protein of the ER, and yes, free glucose is generated inside the lumen. It can then be transported back into the cytosol.

Why use PEPCK in the mitochondria to convert oxaloacetate to PEP, versus that of PEPCK in the cytosol which requires a transporter to allow oxaloacetate to exit the Mitochondrial Matrix before PEP conversion via PEPCK? Is there regulation for this or does the cell use PEPCK in the mitochondria until it exhausts the supply and then is forced to export excess oxaloacetate out of the matrix?

One factor is the availability of the starting material (pyruvate or lactate). Another factor is NADH need/availability. Remember if we use cytosolic PEPCK, and the malate dehydrogenase reaction, oxaloacetate is reduced to malate in the matrix oxidizing an NADH molecule in the process. Then in the cytosol malate is re-oxidized by MDH producing NADH in the process. Thus, this pathway uses NADH in the matrix and produces it in the cytosol.

Can you clarify what transketolase and transaldolase do?

Both transketolase and transaldolase transfer a group from a ketose to an aldose. The difference is that transketolase transfers a two carbon group (hydroxyacetyl) and transaldolase transfers a three carbon group (dihydroxyacetone). If you count up the carbons of the two sugars you start with, you should see a transfer of two or three carbons from the ketose to the aldose depending on which enzyme you use.

I think it is weird that Pyruvate adds a carboxyl group to become oxaloacetate only to immediately drop it to become PEP.

Remember the purpose of pyruvate carboxylase and phosphoenolpyruvate carboxykinase is to overcome the free energy difference between PEP and pyruvate in gluconeogenesis. Since this is no easy task, it is not surprising that it takes two enzymes and the hydrolysis of two high energy bonds from ATP and GTP. So, not totally weird.

For the non-oxidative reaction that yields glyceraldehyde-3-phosphate and fructose-6-phosphate the enzyme used is transketolase right? What holds the two-carbon group? Is it the schiff base shown right before it?

It is again transketolase which requires thiamine pyrophosphate and does *not* form a Schiff base intermediate. Transaldolase (the enzyme right before), which carries a three carbon group (dihydroxyacetone), utilizes a Schiff base intermediate that forms on a lysine side chain from the enzyme.

What is the process by which the transaldolase and transketolase become active? (What I'm asking is, do the enzymes themselves detect some sort of lack, or are the enzymes signaled by another process, or what?)

These enzymes are close to equilibrium and are easily reversible (can go both ways) depending on the needs of the cell (the concentration of reactants and products).

In the pentose phosphate pathway, if the cell only needs ribose, how does it use transketolase or transaldolase to convert fructose-6-phosphate and G3P back to ribose-5-phosphate or vice versa? What is the mechanism?

All of the reactions in the non-oxidative branch are reversible. Glucose is converted to fructose-6-phosphate and glyceraldehyde-3-phosphate by glycolytic enzymes. These molecules are then used by TK and TA (and the epimerase and isomerase) to generate the pentoses.

On the last page of the pentose phosphate pathway lecture, you said that the reducing environment keeps the iron in hemoglobin reduced. Can you clarify?

The iron ion in hemoglobin needs to be in its reduced (Fe^{2+}) form to be able to properly bind and release O_2 . When oxidized to Fe^{3+} , the iron does not bind O_2 .

Why would fructose/galactose enter or be utilized in glycolysis?

We eat them, so they need to be metabolized. It is much better, if possible, to convert a metabolite into an intermediate in an existing pathway, rather than have a whole new pathway designed to specifically metabolize, say galactose.

In Glycolysis, you wrote that TPI, favors DHAP but in the next reaction you have G3P as the reactant, why would it favor DHAP if you need G3P to go on with glycolysis?

The position of the equilibrium is what it is, and depends on the thermodynamics (no enzyme can change that). This particular equilibrium happens to lie in favor of DHAP. It doesn't matter, however, because as you remove G3P, no matter how small the pool of it is, the equilibrium of $\text{DHAP} \leftrightarrow \text{G3P}$ will adjust to make up for the removal of G3P. Remember – as always - enzymes don't affect equilibrium, just the speed at which it is reached.

In the NADH-Q reductase complex, I am not sure exactly how the electrons are passed between the two types of iron-sulfur clusters on their way to reducing ubiquinone. Could you please explain?

Just think of them as a wire that conducts electricity. They pass electrons by alternating their oxidation state from $\text{Fe}^{2+}/\text{Fe}^{3+}$.

What is the breakdown of ATP generated from glucose?

Here's a breakdown for one glucose going through glycolysis and the TCA cycle using the **glycerol phosphate shuttle** (brain and skeletal muscle). Of course, if we use the **malate/aspartate shuttle** we will pump an additional 8 protons per glucose. This will yield another 2.5 or so ATP's.

Glycolysis:

2 ATP
2 NADH (equivalent to 2 FADH₂ - consequence of being in the cytosol)

Two pyruvate dehydrogenase reactions (PDC):

2 NADH (matrix)

Two rounds of the TCA Cycle:

2 ATP (derived from GTP)
6 NADH + 2 FADH₂ (matrix)

Each matrix NADH can lead to 8 protons being pumped out of the matrix and each FADH₂ (or cytosolic NADH) can lead to 4 protons being pumped.

8 NADH: $8 \times 8 = 64$ protons
4 FADH₂: $4 \times 4 = 16$ protons
80 total protons pumped

$\frac{80 \text{ protons}}{3 \text{ protons per ATP}} \approx 27 \text{ ATP's}$

(you can also say each NADH yields about 2.5 ATP's and each FADH₂ yields about 1.5)

If we add this value to the four ATP's we produced directly we get ~31 ATP's per glucose.

Note: this estimate varies quite a bit from textbook to textbook, but is usually in the range of 30-36 ATP's per glucose. Most textbooks nowadays show complex III pumping 4 protons instead of 2, but the total ATP's produced remains about the same, because the extra protons are offset by the ATP synthase reaction requiring ~3.3 protons per ATP instead of 3. So, it is about the same either way – *just be aware it is different in different books*. The problem lies in the fact that the stoichiometry is very hard to measure precisely. Additionally, the proton gradient is not used exclusively for generating ATP – other transporters take advantage of the proton gradient as an energy source.

In complex 3, is the purpose of cytochrome b simply to help regenerate QH₂?

Essentially, yes, but....

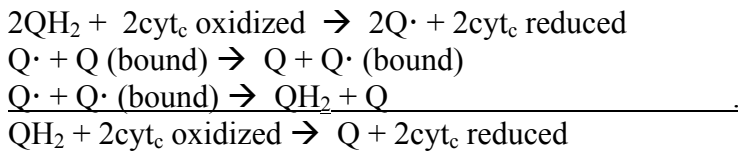
This is not a regeneration of QH₂, rather it is the *disproportionation* of 2 Q·

A disproportionation is a reaction that looks like this:



Notice this generates one Q and one QH₂, and removes two radicals (Q·).

Let's look at the stoichiometry for two electrons going through complex III:

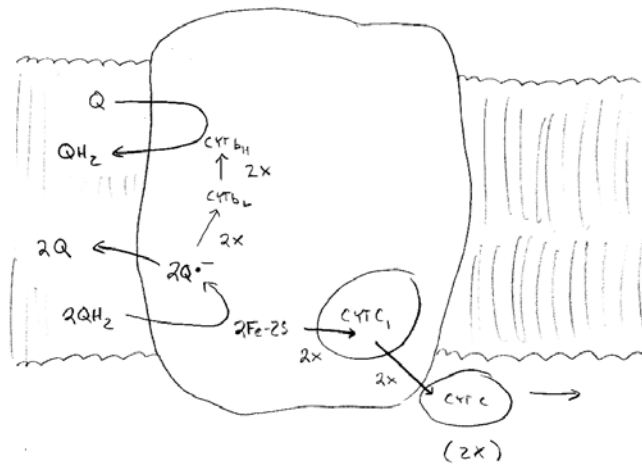


Notice:

- all Q· cancel out
- 1Q cancels out
- 1 QH₂ cancels out

This is how we get the overall equation: QH₂ + 2cyt_c oxidized → Q + 2cyt_c reduced.

I drew the following figure to (hopefully) help illustrate the paths of all the electrons in complex 3:



Every '2x' means that a single electron passes twice (remember 2 cytochrome c proteins are required to carry 2 electrons). The Q reduction occurring on the upper part of the figure represents the 'bound' Q. Try and make sense of the above stoichiometry using this figure.