◉Sheet ○Slides

Subject :	Pentose Phosphate Pathway (PPP)
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Number :	17

Pentose Phosphate Pathway (PPP)

In the last lecture we discussed Pentose Phosphate Pathway; it is composed of two branches which are:
Oxidative reactions, where Glucose 6-phosphate undergo oxidation to give 2NADPH and CO_2 and Ribulose 5-Phosphate, and nonoxidative reactions (The major function of PPP is to produce NADPH and Ribose-5-Phosphate metabolism).

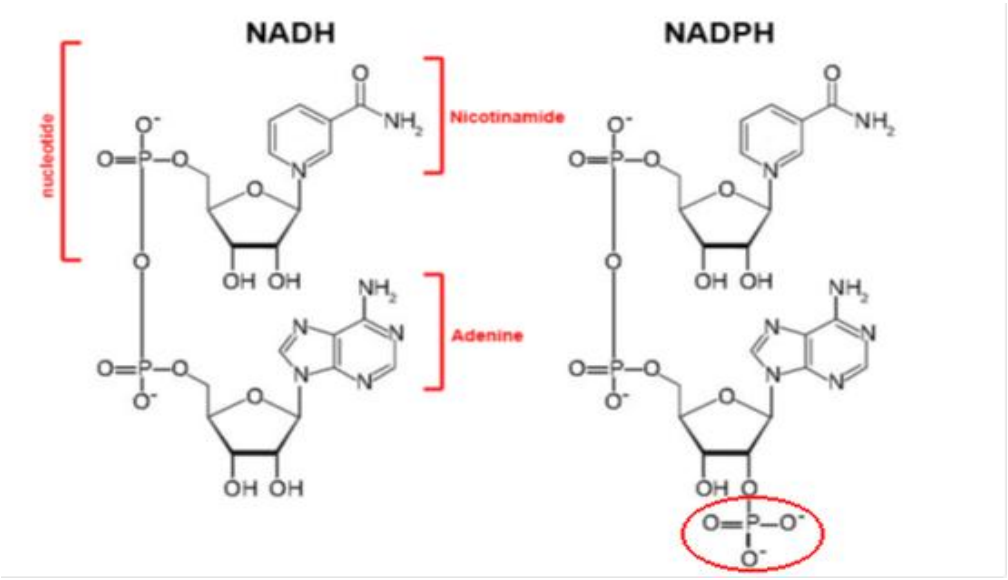
• If the cell requires NADPH and Pentoses, oxidative branch will provide both then we stop here (REMEMBER: It is irreversible phase and active in certain cells).
• If the cell requires only NADPH and does not need Ribulose 5-Phosphate, it can be converted so 6 molecules of Ribulose 5-Phosphate will give 5-molecules of Glucose 6-Phosphate through the nonoxidative branch. (Remember we started with 6 molecules of Glucose 6-phosphate and removed CO2 out of each to make 6 molecules of Ribulose 5-Phosphate, and then they are converted to 4 molecules of Fructose 5-phosphate and 2 molecules of Glyceraldehyde 3-phosphate and then by gluconeogenesis to 5 molecules of Glucose 6-phosphate; this means we lost one Glucose 6-phosphate as 6CO2 to produce 12NADPH).

Q/ what is the name of the sugar produced when Ribose 5-phosphate reacts with xylulose-5-phosphate?
Sedoheptulose 7-phosphate

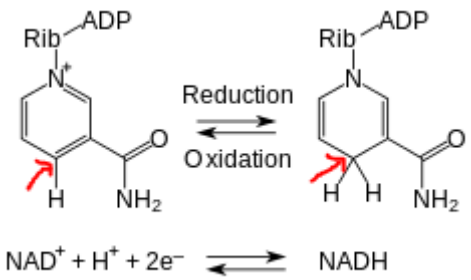
Q/ what is the name of the sugar produced when Erythrose 4-phosphate reacts with xylulose-5-phosphate?
Fructose- 6- phosphate

* In this sheet we will discuss **NADPH** and <u>its Uses.</u>

NADH vs. NADPH



- They both consist of two nucleotides which are composed of nitrogenous bases (Nicotine amide for one nucleotide and Adenine for the other), five-carbon sugars (Ribose), and phosphate groups.
- They both are electron carriers; they participate in Oxidation-Reduction reactions by accepting and donating electrons (in the form of H^-).



As you've noticed, they are very similar in structure; they differ only in the presence of a phosphate group in NADPH (shown in the first figure), so they have almost the same reduction potential, so why not having only one of them instead of both?

1. Enzymes can distinguish them and specifically use one but not the other. Lactate dehydrogenase and Pyruvate dehydrogenase for example can use NAD only, while Glucose 6-phosphate dehydrogenase can use NADP.
2. NADH mostly exist in the oxidized form (NAD^+) and is ready to oxidize, while NADPH mostly exist in the Reduced form(NADPH) and is ready to reduce, so they have different roles; NADH is involved in Oxidative Phosphorylation to produce ATP (involved in oxidation reactions), while NADPH is involved in reduction during biosynthesis (involved in reduction reactions).

To make it easier, imagine the oxidized state as an empty container (empty of electrons) and the reduced stated a container full of electrons. Can we have a high percentage of the full containers and the empty ones of the same containers at the same time? No, but we can have two separate containers one is always empty (oxidized - NAD^+) and the other always full (reduced – NADPH).

In the cytosol of the hepatocytes $[NADP^+]/[NADPH]=1/10$ →NADPH is more predominant than $NADP^+$, but $[NAD^+]/[NADH]=1000$ → NAD^+ is more predominant than NADH. So, Glycolysis, requiring an <u>oxidized form,</u> is always active ; and biosynthesis, requiring a <u>reduced form</u>, is also active at the same time.

So logically, if the enzyme needs an oxidized form (to oxidize another compound) it will use the predominant NAD^+ rather than $NADP^+$; if it needs a reduced form it will use NADPH.

Notice that they only differ in one phosphate group; however, it is enough for enzymes to distinguish between them and to have different roles.

To sum up,

- NADH usually exists in the oxidized form (NAD^+) and is used to oxidize.
- NADPH usually exists in the reduced form (NADPH) and is used to reduce.
- Enzymes used for oxidizing other compounds selectively use NAD^+.
- Enzymes used for reducing other compounds selectively use NADPH.

NADPH is used for reductive biosynthesis *like fatty acid or steroid synthesis*

Some biosynthetic reactions require high energy electron donor to produce reduced products, so we need substance with high reducing ability low reduction potential to be able to donate electrons, and that applies to NADPH and NADH; electrons can move from them to an oxygen molecule a produce high amount of energy.

During fatty acid synthesis:
8 molecules of Acetic Acid (2-carbon molecule , part of **Acetyl-CoA**) will be converted to one molecule of Palmatic Acid (16 carbons); Palmatic Acid is more reduced and at a higher energy state, so we need energy to come from a high energy compound, which is NADPH.

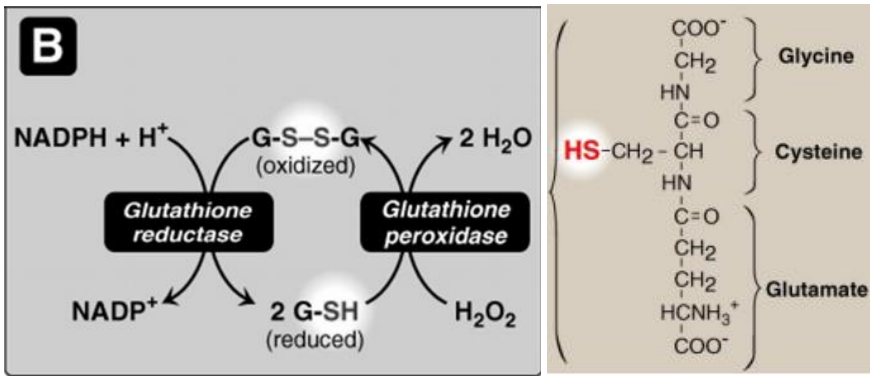
Note: Palmatic Acid ($CH_3(CH_2)_{14}COOH$) is more reduced than Acetic Acid (CH_3COO). **WHY?!** Because the ratio of Hydrogen atoms to Oxygen atoms is **higher**!

NADPH is used in the reaction of reducing Hydrogen Peroxide

Hydrogen Peroxide (H_2O_2) is a compound of the reactive oxygen species (ROS) that are very reactive strong oxidants and thus can cause damage to the DNA, proteins, Lipids or other components of our cells, which leads to cell death, disease or cancer; however, they are being produced continuously as <u>byproducts</u> of aerobic metabolism – Interaction with drugs and environmental toxins (we are not trying to produce them but they are inevitably produced as byproducts).

So we need an anti-oxidant to reduce H_2O_2; and that's what Glutathione **GSH** does. (Glutathione is a tripeptide composed of gama-Glutamic Acid , Glycine , and Cysteine). It protects our body from damage that caused by oxidants (It undergoes into oxidation in order to reduce those agents and protect the cellular components from being oxidized and so damage . These oxidants are usually <u>free radicals).</u>

At the side chain of Cysteine we have a sulfhydryl group **–SH**, so GSH can be oxidized in the presence of **Glutathione Peroxidase** by losing this Hydrogen (and reduce an oxidant like H_2O_2. Now when two molecules of Glutathione lose their Hydrogen, they will be connected by Disulfide Bridge producing **GSSG** (the oxidized form). This oxidized form is no longer active (yet not harmful) , so we need to transform it back again to the reduced form(active form) , using NADPH in the presence of **Glutathione Reductase**. So we eliminated H_2O_2 using GSH and <u>regenerated</u> **GSH <u>using NADPH.</u>**



• Glutathione peroxidase is <u>Selenium</u> metal requiring enzyme, so Selenium is considered a trace element (a chemical element required in our body only in very little amounts); but without this low amount of

Selenium , we can't survive because Glutathione Peroxidase won't function and oxidants will cause huge damage to cells.

• **Superoxide dismutase:** is an enzyme that transforms the strong oxidant Superoxide $\mathbf{O_2^-}$ (which is an oxygen molecule that has accepted one electron) to H_2O_2, and then we can get rid of it as mentioned.

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

• **Catalase**: also can get rid of H_2O_2 as in this reaction:

$$\left(2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2\right)$$

• We have antioxidant chemicals that get oxidized by ROS (instead of cell components) without the need of enzymes, such as: Vitamin E, Vitamin C and Carotenoids. Food that is rich in antioxidant (like flavonoid which is highly rich in antioxidants) is thought to protect from cancer or chronic diseases; but we noticed that supplementation of pure vitamin E or C is not always protective.

(NOTE: One cigarette contains around 400 free radicals and oxidizing compound which damage your DNA, your proteins and non-matured lipids and so affect your body tissues like neurons and other tissues. Normally, our body produces free radicals and ROS but in small amount that our enzymes have the capacity to remove them from our body, so increasing them will cause damage to our tissues even by drugs, toxins, chemical pollutions or smoking).

>> **Sources of ROS**

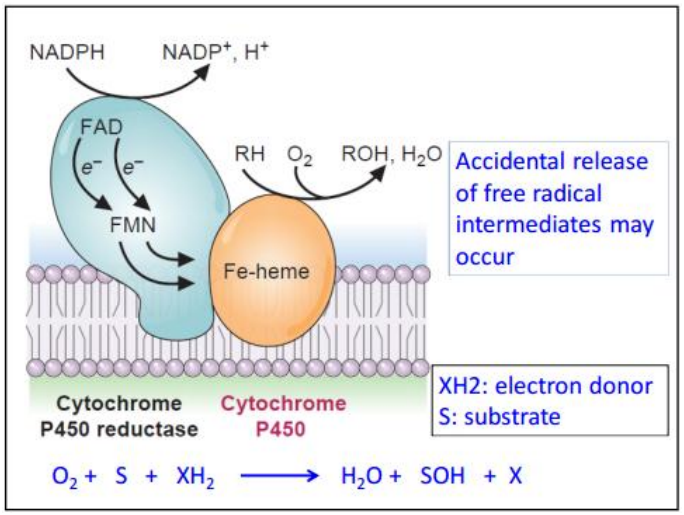
1) **Oxidases**: Enzymes that Catalyze hydrogen transfer from the substrate to molecular oxygen producing hydrogen peroxide as a byproduct.

2) **Oxygenases**: catalyze substrate oxidation by molecular oxygen (it adds oxygen); They are either monooxegenases that transfer one oxygen atom and reducing the other to water, or dioxygenases that transfer two oxygen atoms (used in synthesis of prostaglandins and will be discussed later).

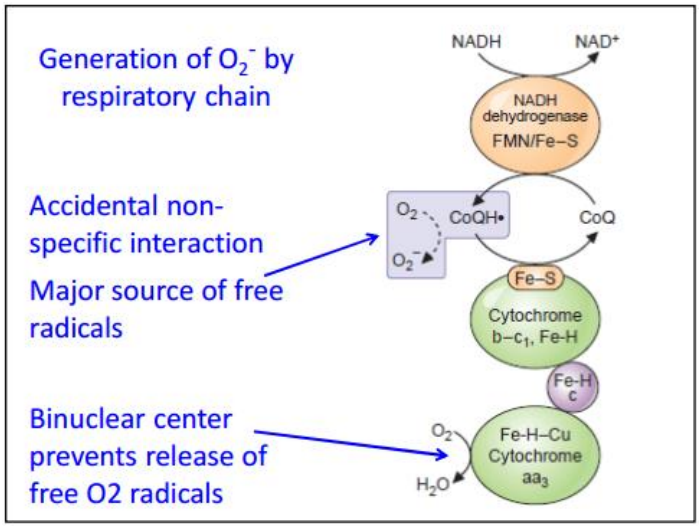
>>NOTE: The difference between oxidases and dehydrogenases that both of them transfer hydrogens, but in dehydrogenases what accept these hydrogens are NAD+ and FAD and in oxidases the oxygen is the acceptor. Oxidases transfer hydrogens to molecular oxygen, but oxygenases transfer oxygen to the substrate – even one oxygen or two oxygens).

3) **Co Enzyme Q in the respiratory chain:** produce many free radicals especially superoxide.
4) **Ionizing radiation:** γ or X.
5) **Peroxidase:** is also produces hydrogen peroxide which is responsible to produce the deadly hydroxyl free radical in the presence of iron (excess iron could be catalyst for reactions to produce free radicals like hydroxyl free radical).
6) **Respiratory Burst (during phagocytosis):** O_2, H_2O_2, $OH^{\bullet}$, NO, $HOCl^{\bullet}$
7) **Cytochrome P450:** superfamily of structurally related mixed function enzymes. (*Chrome means it is **colored*** /red because it contains a Heme group); we have two systems for cytochrome P450:-
   - Mitochondrial system: to hydroxylase steroids, Bile acids and Vitamin D.
   - Microsomal system: for detoxification of foreign compounds; Cytochrome P540 adds hydroxyl groups to make it more soluble and be eliminated.

   $R\text{-}H + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$



In the figure above, notice that Cytochrome P450 reductase contains FAD and FMN like the electron transport chain, so electrons move from NADPH to FAD to FMN and then to the Fe-heme, which will activate Oxygen; one Oxygen atom r gets reduced to H_2O, while the other Oxygen atom becomes incorporated in ROH. Now suppose that the Oxygen molecule received one electron and did not continue, Super Oxide will form, so it is accidental! Other free radicals may be released also.



The figure above shows the respiratory chain; 4 electrons pass the chain to reach O_2 reduce it to $2H_2O$; CoQ may accidently pass an electron to O_2 to form O_2^-.

CoQ is a major source for free radicals (in the binuclear center no free radicals are formed).

>> G6PD Deficiency (Glucose 6-Phosphate Dehydrogenase Deficiency)

G6PD is an Enzyme that catalyze the oxidation of Glucose 6-Phosphate to 6-Phosphogluconate as the first step in the oxidative branch in PPP. G6PD deficiency is very important as it is very common (200-400 million individuals around the world have G6PD deficiency); it is even more common in Middle East.

G6PD deficiency will reduce the production of NADPH, which in turn will affect the activity of antioxidant such as glutathione which depends on NADPH to return to its active state after removing the free radicals. So this will cause the power of antioxidation to be reduced and so the free radicals will find a furtile field to work and damage the intracellular components.

• X-linked recessive Genetic disorder:
This means that it is less common in females because both X chromosomes should have the mutations that cause the deficiency; while in male (XY) we need mutations in the only one X chromosome. Females can be carrier to the disease, so there will be 50% possibility of her male children to have the disease, but 0% for females except if her husband already has the disease (females need the two genes to be affected, but males need only one gene to be affected – remember that female is XX genotype and male is XY genotype-).

• More than 400 different mutations cause G6PD.

• Deficiency provides resistance to Falciparum malaria which is fatal.

>>We will continue G6PD deficiency in the next sheet
>>Don’t forget to return to the doctor’s slides.

A Quick Revision,
not sufficient but just to remember the main concepts.

- NADH (Necotinamide adenine dinucleotide) and NADPH are very similar; they differ in the presence of on phosphate group.
- NADH usually exists in the oxidized form (NAD^+) and is used to oxidize (in oxidative phosphorylation for example).
- NADPH usually exists in the reduced form (NADPH) and is used to reduce.
- Enzymes selectively use one but not the other according to its functions (NAD^+ → if it is used to oxidize; NADPH if it is used for reduction).
- NADPH is used in reductive biosynthesis (such as fatty acids or steroids synthesis) as they need a high energy electron donor to reduce other compounds.
- NADPH is used maintaining of Glutathione in the reduced form.
  a) GSH reduces H_2O_2 to $2H_2O$ and gets oxidized and linked to another oxidized GSH by a disulfide bridge to form GSSG → Glutathione peroxidase.
  b) NADPH reduces GSSG back to GSH so it is functional again → Glutathione Reductase.
- Glutathione peroxidase is Selenium (trace element) requiring enzyme.
- Superoxide dismutase transforms Superoxide $\mathbf{O_2^-}$ to H_2O_2, and then we can get rid of it as mentioned.
- Catalase also get rid of H_2O_2 ($2H_2O_2 \rightarrow 2H_2O + O_2$).
- Antioxidants get oxidized by ROS without enzymes (Vitamin E, C, Carotenoids).
- Sources for ROS: 1)oxidases → H_2O_2 byproduct 2)oxygenases 3+4)CoQ and cytochrome P450 → accidentally pass an electron to O_2 → O_2^- 5)ionizing radiations 6)peroxidases 7)Respiratory burst
- G6PD deficiency → reduce the production of NADPH → free radicals will cause damage.
  -X linked -it is common especially in the middle east –causes resistance to Falciparum malaria.

The life is full with challenges. Challenge yourself to beat other challenges

وأخيرا شيت قصير
😜😜

GOOD LUCK