Pentose phosphate pathway (hexose monophosphate pathway / 6-phosphogluconate pathway)

Overview

-Occurs in the **cytosol**

- includes three IRREVERSIBLE reactions, 2 of them are **oxidative** reactions

- followed by a series of reversible sugar phosphate inter-conversion reactions

- 2 NADPH are produced for each glucose-6-phosphate entering the pathway

- 1 CO_2 formed from carbon 1 of glucose-6-phosphate

-rate and direction of pathway is determined *by amount of intermediates available and needed*

- this pathway produces a major portion of the body's needed NADPH supply (biochemical reductant)

- produces ribose-5-phosphate, required for biosynthesis of nucleotides

- metabolic use of five-carbon sugars obtained from diet or degradation reactions

1) Irreversible reactions: steps 1-3

Important in :-

- Liver, lactating mammary glands, adipose - active in NADPH-dependent biosynthesis of fatty acids
- Testes, ovaries, placenta and adrenal cortex - active in NADPH-dependent biosynthesis of steroid hormones
- Erythrocytes - require NADPH to keep glutathione reduced

A) **Glucose 6-phosphate dehydrogenase** (G6PD) catalyzes the first irreversible oxidation **of glucose-6-phosphate to 6-phosphogluconolactone**.

1 NADPH molecule is produced in the first step

This reaction is specific for $NADP^+$ which is an important co-enzyme

Regulation is primarily by this **G6PD reaction**

NADPH is a **potent competitive inhibitor** of the enzyme (ratio of NADPH/NADP+)

Insulin up-regulates the expression gene for G6PD (in well-fed state)

B) **6-phosphoglucolactone** is hydrolyzed **by 6-phosphoglucolactone hydrolase forming 6-phosphogluconate**

^although this reaction is irreversible it is NOT the rate-limiting step

Oxidative decarboxylation of **6-phosphogluconate** is catalyzed by 6-**phosphogluconate dehydrogenase**, **producing a pentose sugar-phosphate**.

In total : ribulose 5-phosphate, CO_2, and 2 Molecules of NADPH are formed from those 3 irreversible reactions. One H_2O molecule is used up

2) reversible reactions: steps 4-8

Occur in all *cell types synthesizing nucleotides and nucleic acids*

Ribulose-5-phosphate can be converted to either **ribose-5-phosphate** - needed for nucleotide synthesis

Or to intermediates of glycolysis - **fructose-6-phosphate and glyceraldehyde 3-phosphate**

Ribulose 5-phosphate ⟷ ribose 5-phopshate (used for nucleic acid synthesis) - step 4

ribulose 5-phosphate isomerase

Phosphopentose epimerase

Xylulose 5-phosphate (step 5)

Transketolase and **transaldolase** are the enzymes used to produce glycolytic intermediates

Transketolase uses the co-enzyme TPP, it catalyzes two steps (6 + 8) in the same way using 2 reactants and forming 2 products per step

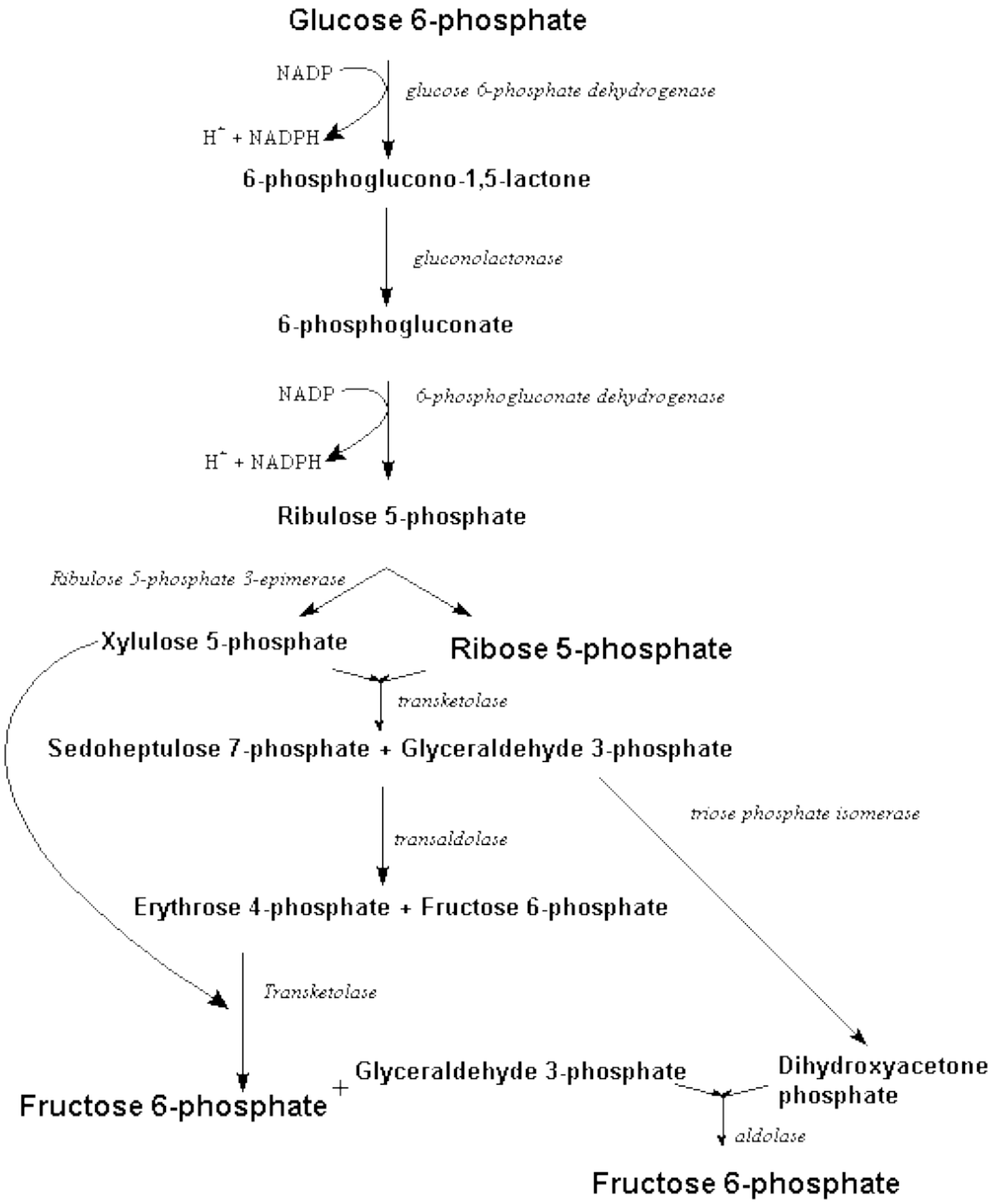
Xylulose 5-phosphate is *converted* to **glyceraldehyde 3-phosphate** by removing 2 Carbons using TPP, those 2 carbons are *added* to **ribose 5-phosphate *forming* sedoheptulose 7-phosphate** (this is step 6)

Transaldolase is an enzyme that removes 3 carbon-units from **sedoheptulose 7-phosphate** (produced in the previous step) *forming* **erythrose 4-phosphate**, those 3 carbons are *added* to **glyceraldehyde 3-phosphate** *forming* **fructose 6-phosphate.**

In the last step (8) : **Xylulose 5-phosphate** is *converted* to **glyceraldehyde 3-phosphate** by *removing* 2 carbons (also using transketolase and its coenzyme TPP) , those 2 carbons are *added* to **erythrose 4-phosphate** *forming* **fructose 6-phosphate** again.

Note: when the demand for ribose conversion to nucleotides and nucleic acids is greater than the demand for NADPH, the non-oxidative reactions can produce ribose 5-phosphate by using the glycolytic intermediates **without** undergoing the 3 irreversible steps.

Summary of the 8 steps :



NADPH

In the cytosol of hepatocytes, the steady-state ratio of $NADP^+$/NADPH is approximately *0.1* which favors the use of NADPH in reductive biosynthetic reactions, while the high ratio of NAD^+/NADH (approximately 100) favors the oxidative role for NAD^+

NADPH is a high-energy molecule, the electrons are used in reductive biosynthesis rather than transfer to oxygen by NADH, part of the energy of glucose 6-phosphate

is conserved in NADPH (has a negative reduction potential value) so it can be used in reactions requiring an electron donor.

Functions of NADPH :

1) Detoxification:

- Reduction of oxidized glutathione
- Cytochrome p450 monooxygenases

2) Reductive synthesis:

- Fatty acid synthesis
- Fatty acid chain elongation
- Cholesterol synthesis
- Neurotransmitter synthesis
- Nucleotide synthesis

Reduction of oxidized glutathione

Glutathione is a tripeptide thiol made from glycine, cysteine and glutamate.

Reduced glutathione can get rid of H_2O_2 by using the enzyme glutathione peroxidase and converting it into 2 molecules of water.

Note: the enzyme glutathione peroxidase requires selenium as a co-enzyme

The oxidized glutathione needs to be regenerated, and this is done by NADPH which works with glutathione reductase to form $NADP^+$ and reduced glutathione again

NADPH here *indirectly* provides electrons for the reduction of H_2O_2

H_2O_2 is formed from the partial reduction of O_2, it is a member of the ROS family, which when produced excessively and in large amounts, damage DNA, proteins (Pro,his,arg,cys,met are the most susceptible amino acids) , and unsaturated lipids.

ROS are produced during normal metabolism in small amounts, and reactions by drugs and environmental factors.

3-5% of consumed O_2 is converted to ROS

O_2^- , H_2O_2, and OH^- all can cause cell injury

Formation of OH radicals

1- The Fenton reaction

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^{+} \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + HO^{\bullet} \quad (2)$$

$$HO_2^{\bullet} \leftrightarrow H^{+} + O_2^{\bullet -} \quad (3)$$

$$Fe^{3+} + HO_2 \rightarrow Fe^{2+} + H^{+} + O_2 \quad (4)$$

2- The Haber-Weiss reaction

$$O_{2\text{-}} + H_2O_2 \longrightarrow O_2 + OH\bullet + OH^{-}$$

Diseases associated with ROS injury:

- Atherosclerosis
- Respiratory diseases
- Cancer
- Diabetes
- Liver Damage
- Motor neuron disease
- Aging

*oxidase enzymes are confined to sites equipped with protective enzymes

There are various intracellular reducing agents such as ascorbate, vitamin C & E, and B-carotene, those have been correlated with the decreased risk of certain cancers, as well as frequency of certain chronic health problems.

Primary antioxidants - antioxidant enzymes

Superoxide dismutase, catalse and GSH peroxidase

Secondary antioxidants:

A) Dietary

- Vitamin E (tocopherol) (most widely distributed antioxidant_

- Vitamin C
- B-carotene
- And some others

B) endogenous antioxidants

C) repair mechanism of DNA, oxidized fatty acids of membrane lipids and oxidized amino acids

D) compartmentation e.g. peroxisomes

Flavonoids (polyphenolic compounds), present in green tea, chocolate, red wine and fruits skin (e.g. onions)

Possible functions:

- Inhibition of O_2^- production
- Free radical scavenger
- Chelate Fe + Cu
- Maintenance of Vitamin E

Endogenous antioxidants

- Uric acid
- GSH
- Melatonin
- Bilirubin
- Lipoic acid
- Ub(coQ)

Some Flavonoids:

- Catechins: strawberries, green + black tea
- Kaempferol: brussel sprouts of apple
- Quercetin: beans, onions, apples, and fruit skin
- Epicatchin: cocoa, red wine

Vitamin antioxidants: chain-breaking, terminate free radical lipid peroxidation

Vitamin E donates a single e

Carotenoids accept an electron from lipid peroxy radicals. (rich in dark green leafy vegetables

Vitamin C accepts a single e from O_2^- . H_2O_2, OH^-, HOCL and peroxyl radicals, it also regenerates the reduced form of Vitamin E

Cytochrome p450 monooxygenase system (mixed function oxygenase)

A superfamily of structurally related monooxygenases, 100 different isoenzymes in humans with overlapping specificities.

O_2^- radicals can be accidently formed in the ETC at the site of CoQH where O_2 will be partially reduced to O_2^-.

Fe-Cu Cytochrome in the 4th complex is the main site for the prevention of the release of free O_2 radicals

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

R can be a steroid, drug or a chemical

1- Mitochondrial system : In steroidogenic tissues such as placenta, ovaries, testes or adrenal cortex, this system functions in the biosynthesis of steroids, it is used to hydroxylate intermediates in the conversion of cholesterol to steroids, this process makes the hydrophobic compounds more water-soluble.

The same system is used in the liver for the synthesis of bile acids, and for the hydroxylation of cholecalciferol to Vitamin D3.

It is also used in the kidney to hydroxylate vitamin D3 to its biologically activated form.

2- Microsomal system: important in the detoxification of foreign compounds within the smooth ER (particularly in the liver)

Toxins can be hydroxylated using NADPH as a source of reducing equivalents

This modification is done for 2 purposes :

Firstly to activate or inactivate a drug, or secondly to make a toxic compound more soluble facilitating its excretion

Also usually the new hydroxyl group will serve as a site for conjugation (phase 2 reactions) in order to increase the solubility

Phagocytosis by white blood cells

Neutrophils and monocytes are armed with both oxygen-independent and oxygen-dependent mechanisms for killing bacteria by phagocystosis

1- oxygen-independent mechanism: uses pH changes in phagolysosome and lysosomal enzymes to destroy pathogens

2- oxygen-dependent mechanism: NADPH oxidase and myeloperoxidase (MPO)

After a bacteria has been recognized and engulfed, NADPH oxidase assembles itself and is activated, it reduces oxygen into superoxide O_2^- (the rapid consumption of oxygen is known as respiratory burst)

Electrons move from NADPH to O_2 via FAD and heme, generating O_2^- radical.

Genetic deficiency in NADPH oxidase cause chronic granulomatous diseases

Next superoxide is converted to H_2O_2 either spontaneously or by superoxide dismutase.

In the presence of MYO, a heme-containing lysosomal enzyme in the phagolysosome converts H_2O_2 into HOCL hence killing the bacteria.

The peroxide can be partially reduced to hydroxyl radical or fully reduced to water by catalse or glutathione peroxidase

Synthesis of Nitric oxide:

Arginine, **O_2, NADPH** are substrates for the *cytosolic NO synthase enzyme*, **FMN, FAD, heme and tetrahydrobiopeterin** are co-enzymes**, NO and citrulline** are the products of this reaction

3 NO synthesases can be found, 2 of them are constitutive (they are synthesized at a constant rate regardless of physiological demand) they are also $ca2^+$calmodulin dependent enzymes

They are found in

1) the endothelium eNOS - isoform III

2) neural tissue nNOS - isoform I

- Constantly producing low levels of NO

The third inducible enzyme is iNOS (isoform II), it is expressed in many cells, including hepatocytes, macrophages, neutrophils and monocytes.

Some inducers include TNF-a , bacterial endotoxins and inflammatory cytokines, those are found to induce the production of large amounts of NO over hours or even days. Bacterial LPS and y-interferon release stimulate the enzyme synthesis in response to infection, activated macrophages form superoxide radicals that combine with NO to form intermediates that decompose producing highly bactericidal OH radical

NO is a potent inhibitor of platelet aggregation by activating cGMP pathway, it is also characterized as a neurotransmitter in the brain.

Actions of NO on vascular endothelium: after it is synthesized in endothelial cells by eNOS, it diffuses to vascular smooth muscle where it activates the cytosolic form of guanylate cyclase to produce cGMP - guanylate cyclase is not membrane associated unlike adenylate cyclase -

Rise in cGMP causes activation of protein kinase G which phosphorylates $Ca2^{+}$ channels causing the decrease of $Ca2^{+}$ entry into smooth muscles, this decreases $Ca2^{+}$ - calmodulin activation of myosin light chain kinase, thereby decreasing smooth muscle contraction.

Vasodilator nitrates, such as nitroglycerin and nitroprusside, are metabolized to NO to lower blood pressure

Sildenafil citrate, used in the treatment of erectile dysfunction inhibits the phosphodiesterase that inactivates cGMP

Glucose 6-P dehydrogenase deficiency

- inherited disease

- characterized by anemia due to inability to detoxify agents

- most common disease-producing enzyme abnormality in humans (> 400million worldwide)

- highest prevalence in middle east, tropical Africa, Asia and Mediterranean

- X-linked, mutations in gene coding for G6PD

- only some exhibit symptoms; neonatal jaundice from increased production of unconjugated bilirubin

-chronic hemolysis shorts life span

- healthy carriers show increased resistance to falciparum and malaria (females)

- more than 300 different mutations, most of them are missense and point, no frame shift or large deletion

- complete deficiency is lethal

In red blood cells; less NADPH is formed → harder to maintain glutathione reduced → decrease in cellular detoxification → more free radicals and peroxides formed → sulfhydryl groups in proteins are oxidized → denatured proteins → insoluble masses (Heinz bodies) attach to red cell membranes.

Furthermore, oxidation of membrane proteins causes the red cells to be more rigid, so they are removed from the circulation by macrophages in the spleen and liver. G6PD deficiency is most severe in erythrocytes since the pentose phosphate pathway is the only pathway occurring there which generates NADPH.

Other tissues have alternative sources for NADPH production such as $NADP^+$-dependent malate dehydrogenases.

Factors affecting G6PD deficiency:

Most individuals are asymptomatic, however some patients developed hemolytic anemia if they are treated with an oxidant drug, ingest fava beans or contract a severe infection.

1- oxidant drugs : AAA ; Antibiotics, Antimalarials, and antipyretics

2- Favism: Mediterranean variants are the most susceptible. Fava beans contain purine glycosides as vicin and isouramil.

3- infections are the most common precipitating factors as inflammatory responses produce free radicals

Almost all G6PD variants are caused by point mutations in the gene for G6PD, the structure of the enzyme's active site is not affected and hence the enzymatic activity

isn't affected either. However, many mutant enzymes show altered kinetic properties, such as decreased catalytic activity, decreased stability, or an alteration of the binding affinity for $NADP^+$, NAPH or glucose 6-phosphate.

Variants can be classified according to the severity of the disease:

Class	Clinical Symptoms	Residual Enzyme activity	NOTES
I	Very severe (chronic hemolytic anemia)	<10%	rare
II	Severe (episodic hemolytic anemia)	<10%	(Mediterranean) Decreased stability resulting in decreased enzymatic activity
III (G6PD A-)	Moderate	10-60%	RBCs contain an unstable but normal G6PD with most of the enzymatic activity present in reticulocytes and younger erythrocytes
IV	None	>60%	G6PD B, even the oldest RBCS have a sufficient level of activity to provide protection