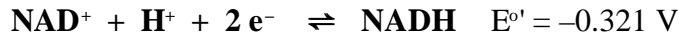


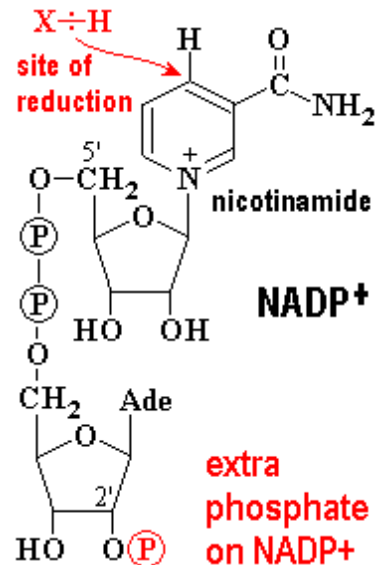
Chem*3560 Lecture 17: Pentose phosphate pathway and NADPH production

NAD⁺ and NADP⁺ have different roles in the cell

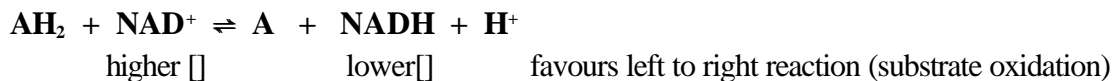
NADP⁺ differs from NAD⁺ by the presence of an **extra phosphate on the 2'-position of adenosine**.



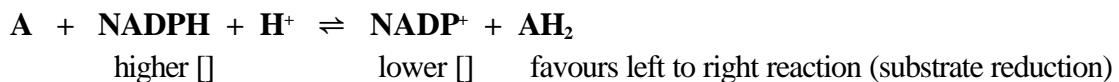
Although this difference is too distant from the site of reduction to have much effect on reactivity, it does allow different enzymes to distinguish NAD⁺ from NADP⁺. Each can play the role of oxidation-reduction coenzyme, but enzymes that use them are specific and selective either for NAD⁺ or for NADP⁺ (Lehninger p.518-519).



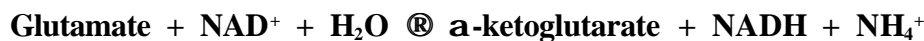
Cells maintain the ratio $[\text{NADH}] / [\text{NAD}^+]$ at low values; 0.1 in mitochondria, and down to 0.002 in cytoplasm. This means that NAD⁺ is in excess, and this version of the coenzyme works best as a hydrogen acceptor in substrate oxidation reactions of catabolic pathways.



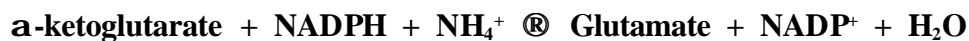
In contrast, the ratio $[\text{NADPH}] / [\text{NADP}^+]$ is kept high (50-100), so that NADPH is in excess, and this favours reactions where NADPH acts as a hydrogen donor for substrate reduction, most frequently in anabolic or synthetic pathways.



Glutamate dehydrogenase provides a good example. The NAD⁺ dependent isozyme is used for glutamate oxidation:



NADP⁺- dependent isozyme is used in glutamate synthesis:



The concentration ratios dictate the reaction direction in each case.

The Pentose phosphate pathway is a major producer of NADPH

Reactions with very negative reduction potential are needed in order to make the required excess of NADPH. Biochemical reactions with the desired reducing power include **oxidation of aldehyde to carboxylate**, and oxidations reactions **coupled to a decarboxylation** for extra driving force. Both of these processes are found in a glucose oxidizing sequence called the pentose phosphate pathway (Lehninger p.558-559).

Pentose phosphate pathway

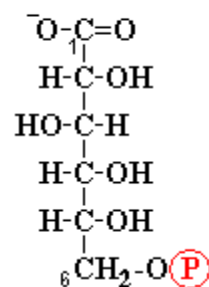
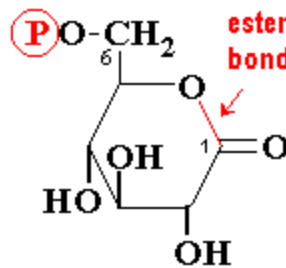
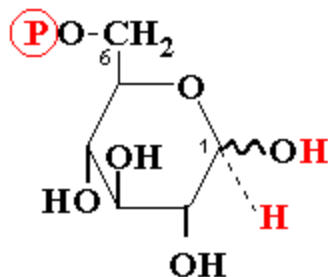
The pentose phosphate pathway has two distinct phases:

- the oxidative phase**, which produces NADPH and the pentose ribulose-5-phosphate;
- the rearrangement phase**, which rearranges pentose phosphates into triose and hexose phosphates, which can be returned to the normal glycolysis sequence.

Glucose-6-phosphate dehydrogenase

Reactions start with *glucose-6-phosphate dehydrogenase*, which oxidizes the aldehyde C-1 of glucose-6-phosphate to a carboxylate group. Glucose aldehyde normally exists a hemiacetal pyran ring, in which the aldehyde has combined with O-5. The product remains as a ring, with the carboxylate at C-1 forming an ester bond to O-5. The carboxylate anion derived from glucose is **gluconate**, and cyclic esters of this type are called **lactones**, hence the product name **6-phosphogluconolactone**.

glucose-6-phosphate dehydrogenase

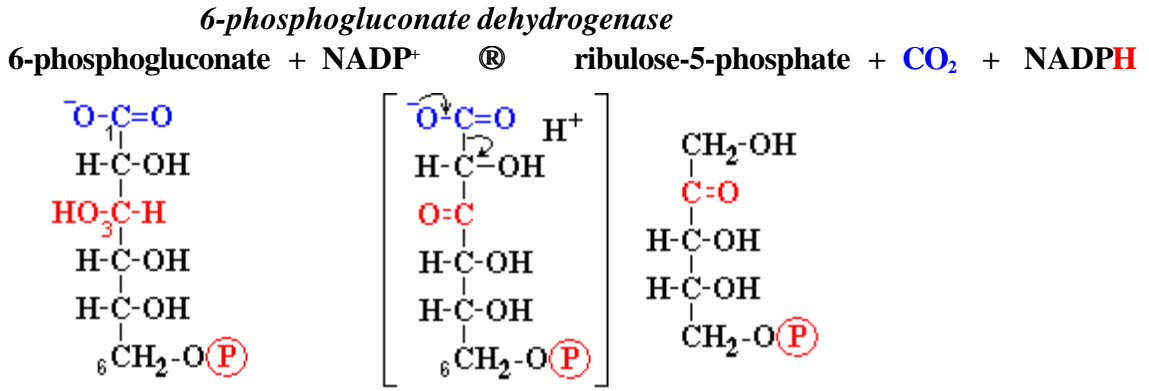


The lactone ring is then hydrolysed to break the ester bond and give the open chain form **6-phosphogluconate**.

6-phosphogluconate

6-Phosphogluconate dehydrogenase

A second oxidation of O-3 to a ketone is coupled to decarboxylation. The product is now the ketone pentose, ribulose-5-phosphate.



Thus the overall yield for the two dehydrogenases is 2 NADPH per glucose-6-phosphate entering the pathway.

Control of pathway activity

The quantity of substrate entering the pathway is controlled by the low concentration of NADP⁺ that remains when excess NADPH accumulates. As soon as any synthetic pathway consumes any NADPH and regenerates more NADP⁺, pentose phosphate oxidation reactions can resume.

Rearrangement phase of the pentose phosphate pathway

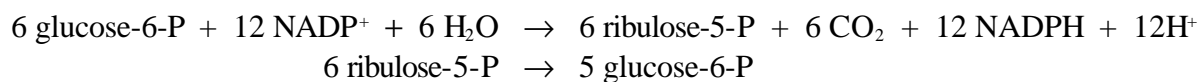
Ribulose-5-phosphate has no immediate uses in the cell, so is rearranged into other forms:

Isomerization to ribose-5-phosphate, which can be used for RNA and DNA nucleotides.

Rearrangement to yield fructose-6-phosphate and glyceraldehyde-3-phosphate;

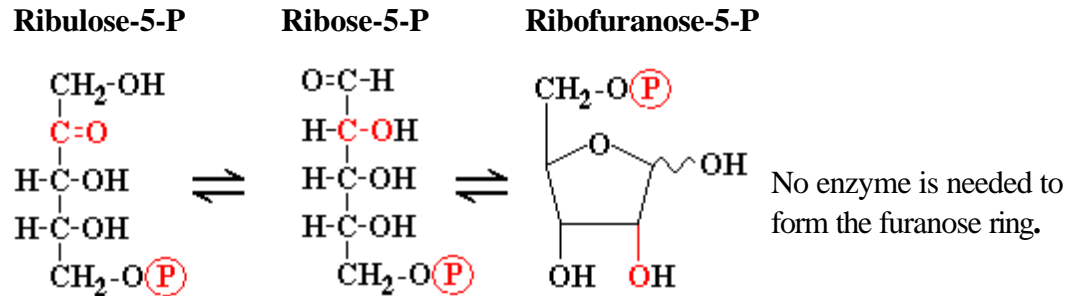
If glycolysis is concurrently active, these intermediates simply enter the glycolysis sequence and are consumed to generate ATP.

If gluconeogenesis is currently active, then fructose-6-phosphate and glyceraldehyde-3-phosphate are converted back into glucose-6-phosphate. Six original molecules of glucose-6-phosphate yield 6 molecules of ribulose-5-phosphate, which rearranges into 5 molecules of glucose-6-phosphate. By cancellation, this is equivalent to total oxidation of 1 molecule of glucose-6-P to 6 CO₂, which yields 12 NADPH

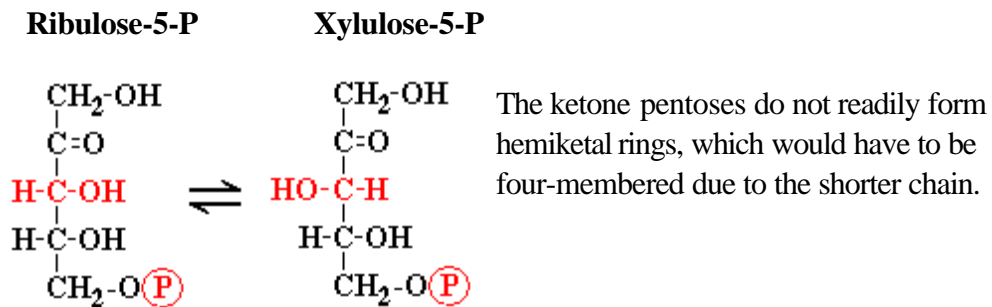


Ribulose-5-phosphate is converted to ribose-5-phosphate and xylulose-5-phosphate

Ribose-5-phosphate is generated by *phosphopentose isomerase*



Xylulose-5-phosphate is generated by *ribulose-5-phosphate epimerase*



The final three reactions involve two enzymes, **transketolase**, **transaldolase**, then **transketolase** again. These two enzymes shuffle groups of two or three carbon atoms around to rearrange the carbon skeleton of the pentoses and so regenerate hexose and triose phosphates. In addition to adjusting chain length, the rearrangements have to **reproduce the carbon configuration** that corresponds exactly to fructose-6-phosphate.