

## Pentose phosphate pathway

- 1 Glucose 6-phosphate (G6P) is usually oxidised by glycolysis or involved in glycogenesis, but it can also be oxidised, thereby entering the *pentose phosphate pathway*. This pathway is important because it provides
  - a the pentoses required for the synthesis of several important compounds including RNA, DNA, ATP (and other NTPs<sup>1</sup>), NADH, FAD and CoA.
  - b NADPH, which is
    - i generally involved in biosynthetic reactions<sup>2</sup> in the place of NADH<sup>3</sup> and
    - ii involved in counteracting the effects of oxygen radicals (especially in erythrocytes and the cells of the lens and cornea, which are directly exposed to O<sub>2</sub>).

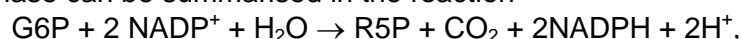
The pathway has two phases:

- i the oxidative phase<sup>4</sup>, in which G6P is oxidised twice to yield ribose 5-phosphate (R5P), a pentose phosphate, and two NADPH, and
- ii the recycling phase, in which unused pentose phosphates are 'recycled' to G6P — in essence, this phase is just a process whereby pentose phosphates are cut up and pasted back together in 6 carbon units.

The enzymes of the pentose phosphate pathway are located in the cytosol, so it interacts with both glycolysis and gluconeogenesis. For example, the three pathways share common intermediates<sup>5</sup>.

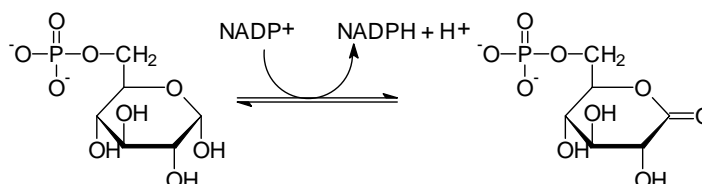
### 2 Oxidative phase

The oxidative phase can be summarised in the reaction



but it requires four enzymes.

- a Glucose 6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49) oxidises G6P to 6-phosphoglucono- $\delta$ -lactone<sup>6</sup> (6PGL), using NADP<sup>+</sup> as the electron acceptor.



The G6PDH reaction is an important point of control for this pathway.

- i Since  $Q \cong 6.3 \times 10^{-6}$  (estimated from Mulquiney & Kuchel 1999 *Biochem J* **342**, 581-596) and  $K_{eq} = 1.36$  the reaction tends to proceed in the direction of 6PGL synthesis.
- ii In general, the activity of G6PDH is regulated by [NADP<sup>+</sup>], which activates the enzyme as its concentration rises.
- iii On a longer timescale, the expression of the single X-linked gene encoding G6PDH is increased by

<sup>1</sup> An NTP is a nucleotide triphosphate, so it includes ATP, GTP, UTP and so on.

<sup>2</sup> The pentose phosphate pathway is especially important in the synthesis of fatty acids (in liver and adipose tissue) and of cholesterol and steroid hormones (liver and adrenal gland).

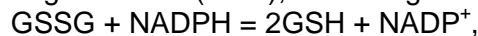
<sup>3</sup> In the cytosol of hepatocytes [NADH]/[NAD<sup>+</sup>]  $\cong 8 \times 10^{-4}$ , but [NADPH]/[NADP<sup>+</sup>]  $\cong 70$ , so the separation of these two pools of reducing equivalents is important for the simultaneous operation of both biosynthetic reactions (involving NADP(H)) and catabolic reactions (involving NAD(H)).

<sup>4</sup> For this reason the pathway is also called the *oxidative* pentose phosphate pathway to distinguish it from the *reductive* pentose phosphate pathway that happens in plants and photosynthetic bacteria.

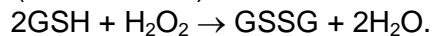
<sup>5</sup> In particular, you should think carefully about the regulation of the metabolism involving G6P.

<sup>6</sup> A lactone is a cyclic ester.

- excess carbohydrate and correspondingly decreased as carbohydrate supplies decline (as are several genes encoding proteins involved in lipogenesis<sup>7</sup>)
    - ⇒ insulin (indicative of high blood glucose) increases the expression of the G6PDH gene
    - ⇒ glucagon (indicative of low blood glucose) or cAMP decrease the expression of the G6PDH gene
  - oxidative stress (such as superoxide radicals and H<sub>2</sub>O<sub>2</sub>)
- iv About 400 million people carry mutations<sup>8</sup> of the gene encoding G6PDH. Affected individuals (in which G6PDH do not work) have a particular problem in coping with oxidative stress and suffer from haemolytic anaemia (because of all the O<sub>2</sub> to which erythrocytes are exposed). Usually, the NADPH that is produced by the oxidative phase of the pentose phosphate pathway reduces oxidised glutathione<sup>9</sup> (GSSG) to reduced glutathione (GSH), according to the reaction

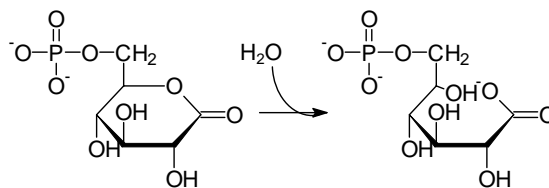


which is important in coping with oxidative stress through the reaction of glutathione peroxidase (E.C. 1.11.1.9)



There are up to six different isozymes of glutathione peroxidase<sup>10</sup>, several of which contain selenocysteine<sup>11</sup>, a modified form of cysteine in which the thiol group (-SH) at the end of the sidechain is replaced with -SeH.

- b Lactonase (E.C. 3.1.1.17) hydrolyses the 6-phosphoglucono- $\delta$ -lactone to 6-phosphogluconate



- c 6-Phosphogluconate dehydrogenase<sup>12</sup> (E.C. 1.1.1.44) oxidatively decarboxylates 6-phosphogluconate to ribulose 5-phosphate (Ru5P). Note that the structures have not been drawn in their cyclic forms, but both the cyclic and acyclic forms of these carbohydrates are found in solution.

<sup>7</sup> While there is a strong connection between lipogenic metabolism and the pentose phosphate pathway, even tissue in which lipogenesis does not occur have the pentose phosphate pathway.

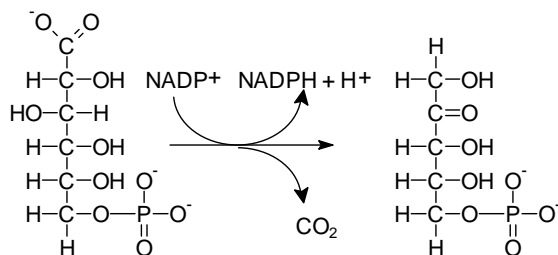
<sup>8</sup> More than 300 different mutations of the G6PDH gene have been reported. Deficiency in G6PDH activity protects against severe malaria, because the growth of the parasite (*Plasmodium* spp.) is reduced by oxidative stress (Min-Oo & Gros2005 *Cell Microbiol* **7**, 753-763).

<sup>9</sup> Glutathione is a tripeptide ( $\gamma$ -glutamate-cysteine-glycine) which is a monomer in the reduced form (GSH) and a dimer in the oxidised form (GSSG). In the dimeric form, there is a disulphide bond between the S atoms at the end of the cysteine sidechains. Glutathione is involved in much of oxidative metabolism, and replacing or modifying any of the three amino acids significantly influences its effectiveness as a substrate in various reactions (Lucente *et al* 1998 *Il Farmaco* **53**, 721-735).

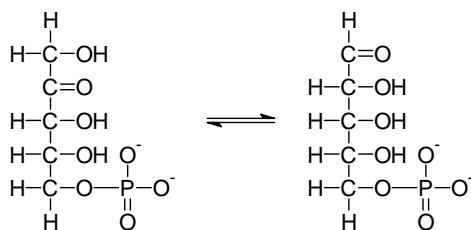
<sup>10</sup> See Arthur (2000 *Cell Mol Life Sci* **57**, 1825-1835) for a review.

<sup>11</sup> As well as selenocysteine, selenomethionine is also present in some proteins. There is an enormous literature on selenium, ranging from the nutritional (Schrauzer 2000 *J Nutr* **130**, 1653-1656) to the more biochemical (Birringer *et al* 2002 *Nat Prod Rep* **19**,693-718).

<sup>12</sup> Because this enzyme also catalyses a reaction involving NADPH, like G6PDH it is also a potential target for anti-malarial drugs (Hanau *et al* 2004 *Current Med Chem* **11**, 2639-2650).



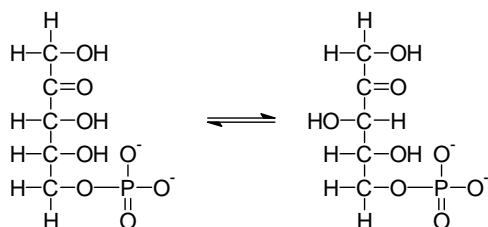
- d Phosphopentose isomerase (E.C. 5.3.1.6) catalyses the conversion of Ru5P to ribose 5-phosphate (R5P).



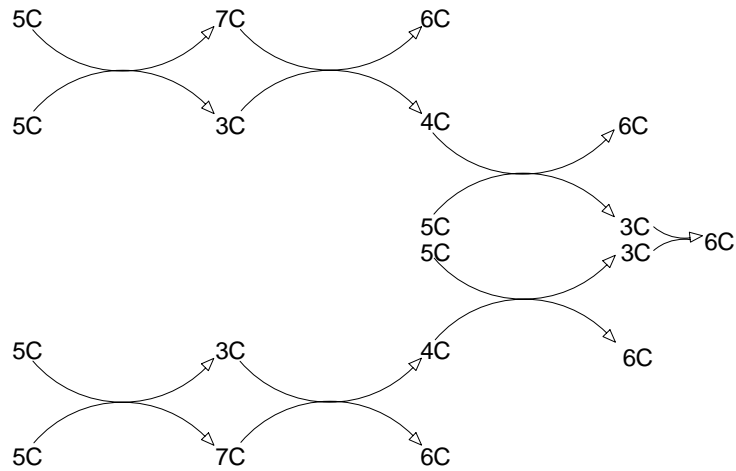
### 3 Recycling phase

The R5P that is formed in the oxidative phase of the pathway is incorporated into various products, but any 'extra' pentose phosphate is recycled back to G6P. This is particularly important in those tissues, such as erythrocytes, that require the NADPH produced by the pathway but have a lesser requirement for R5P.

- a The recycling process is essentially a process of 'cutting and pasting' carbohydrate molecules with different numbers of carbon atoms (figure 1). Obviously, six pentoses (5C) can be combined to yield five hexoses (6C). Each of the 'reactions' is reversible.
- b The pathway requires three enzymes, each of which catalyses a reversible reaction.
- i Ribulose 5-phosphate epimerase (E.C. 5.1.3.1) converts Ru5P to its epimer xylulose 5-phosphate (Xu5P)



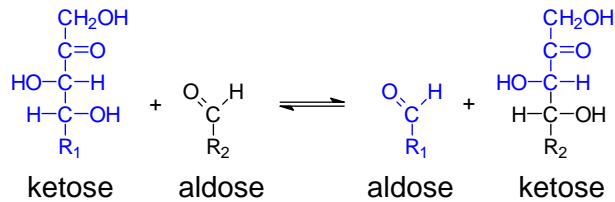
- ii Transketolase (E.C. 2.2.1.1) then moves a two carbon unit from Xu5P (a ketose) to R5P (an aldose) forming a seven carbon aldose (sedoheptulose 7-phosphate, S7P) and glyceraldehyde 3-phosphate (G3P, a triose phosphate).



**Figure 1.** The carbon flow in the recycling phase of the pentose phosphate pathway. Rather than giving the compound names, just the number of carbon atoms in each is indicated.

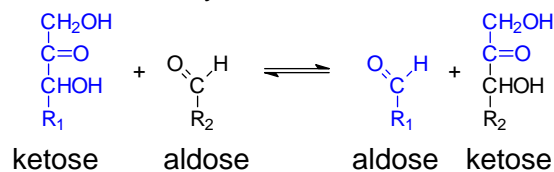
iii Transaldolase (E.C 2.2.1.2) then cuts a three carbon fragment from S7P and links it to G3P yielding F6P and erythrose 4-phosphate (E4P, an aldose). The F6P can enter glycolysis. This reaction is similar to that catalysed by aldolase in glycolysis.

- In general, transaldolase moves a unit similar to dihydroxyacetone to various aldoses (so one non-systematic name for the enzyme is dihydroxyacetone transferase)

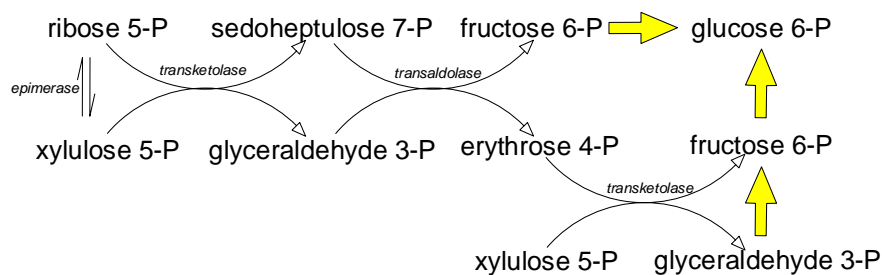


iv Transketolase then moves a two carbon unit from Xu4P to E4P forming F6P and G3P. A second round of the cycle would produce two molecules of G3P which can be converted to fructose 1,6-bisphosphate by aldolase.

- In general, transketolase catalyses the reaction



c The recycling phase of the pathway can be drawn as is shown below (the yellow arrows represent various gluconeogenic reactions).



- 4 Glycolysis and the pentose phosphate pathway.
  - a Since the main regulation of this pathway occurs in connection with G6PDH and the pathway interacts with at least two other pathways, regulation of the pathways around G6P is important.
  - b Recall
    - i G6P and/or F6P is/are allosteric inhibitor(s) of hexokinase (depending on the tissue)
    - ii insulin activates glycolysis, glycogenesis and the pentose phosphate pathway, but reduces gluconeogenesis
    - iii glucagon reduces glycolysis, glycogenesis and the pentose phosphate pathway, but activates gluconeogenesis
    - iv G6PDH is activated by high  $[NADP^+]$  and inactivated at high  $[NADPH]$

*Critical points*

- Supplies
  - pentose phosphates for various compounds
  - NADPH for detoxification (especially glutathione)
- Two phases
  - oxidative phase produces NADPH and pentose phosphates
  - recycling phase salvages pentose phosphates
- Regulation of the pathway is mostly through G6PDH
  - $NADP^+$
  - insulin, glucagon, carbohydrate
- Connection to glycolysis and gluconeogenesis