Amino Acid Biosynthesis

Introduction

- We now consider the **biosynthesis** of **amino acids**, the **basic building blocks** for **cellular constituents**. To do this, cells need:
  - A **carbon sekeleton**.
  - **Reducing power**
  - A source of **organic nitrogen**. This is particularly problematic because even though the earth has an **abundant supply** of **nitrogen**, it is primarily in the form of **inert atmospheric nitrogen**.
  - **Energy** (as ATP).

Obtaining Organic Nitrogen

Nitrogen is obtained and incorporated into NH_2 groups in **three steps**:

1) **Inorganic nitrogen** is **reduced** to **NH_3**.
2) **NH_3** is **assimilated** into **glutamate**.
3) **Transamination** or **carbon skeleton alteration** occurs, and the **NH_2** group is transferred to other amino acids.

Stage I – Getting NH_3

In the first step, NH_3 has to be obtained for incorporation into amino acids. Nitrogen is present on earth in ***two*** forms – in the atmosphere as **N_2** and in the soil as **NO_3^-**. Some organisms obtain NH_3 from the former, some form the latter:

- **Fixation of Atmospheric Nitrogen**

  Even though the reaction of **nitrogen** and **hydrogen** to form **ammonia** is **thermodynamically favourable**, it is **difficult kinetically** because the **intermediates** along the reaction pathway are **unstable**.

Higher organisms have lost the ability to fix nitrogen, but some **bacteria** still retain that ability, including several **cyanobacterial** species and the ***rhizobium*** species, which **invade** the **roots** of **leguminous plants** in which they form **root nodules** [in these nodules, the cells are ***stuffed*** with bacteria].

These are responsible for about **60%** of the world's newly fixed nitrogen. **Lightening** and **UV radiation** provides another **15%**, and **25%** is fixed by **industrial processes**. These, however, involve an **iron catalyst**, **500° C** and **300 atm** pressure.

The **fixation** of **atmospheric nitrogen** is carried out by the **nitrogenase complex**. It consists of two proteins:

- ***A reductase (an Fe protein – 4Fe-4S cofactor)*** which obtains **high energy electrons** from **reduced ferrodoxin**.
- ***Nitrogenase (an MoFe protein – tetramer of a_2b_2 and 4Fe-4S cofactor, in ratio 2M0:28Fe:28S***), which **uses** these **high energy electrons** to **reduce N_2** to **NH_3**. This component is **extremely sensitive to inactivation by O_2**. **Leguminous plants** maintain a very **low** concentration of O_2 in their **root nodules** by **binding O_2 to leghemoglobin**.

ATP is used to **transfer electrons** from the **reductase** to the **nitrogenase** (2 ATP per electron – the binding of ATP causes a **conformational change** that moves this part **closer** to the **nitrogenase**, whence the electron can pass from one to the other). This is **not** required to make the reaction **thermodynamically favourable** – rather, it is essential to **reduce** the **heights** of **activation barriers** along the pathway.

Theoretically, this is a **6 electron** process, but the **biological reaction** always generates **1 mol of H_2**. So:

$$N_2 + 8e^- + 8H^+ + 16ATP + 16H_2O \longrightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

- **Reduction of Nitrates in the soil**

  Nitrates are fairly abundant in the soil due to the action of **nitrifying bacteria**. All other plants, and many bacteria, obtain their nitrogen by reducing nitrate (NO_3^-) in two steps:

  1) **Nitrate reductase** contains an **electron transfer chain** of **FAD**, **cytochrome** b_{557} and **Mo**. Depending on the tissues, the **electrons** come from **NADH** or **NADPH**:

  $$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$$

  2) **Nitrite reductase** contains **sirohaem** and a **4Fe-4S** centre. The **reducing power** comes from **NADPH** via **ferrodoxin**:

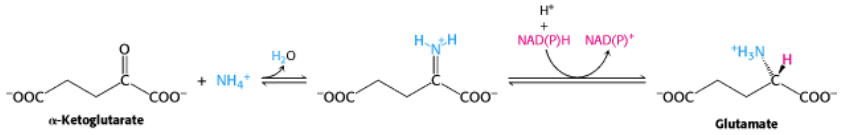
  $$NO_2^- + 8H^+ + 6e^- \longrightarrow NH_4^+ + 2H_2O$$

Stage II – Incorporating NH_3 into glutamate

The NH_3 group is incorporated into either **glutamate** or **glutamine**:

- **Incorporation into glutamate [animals and fungi]**

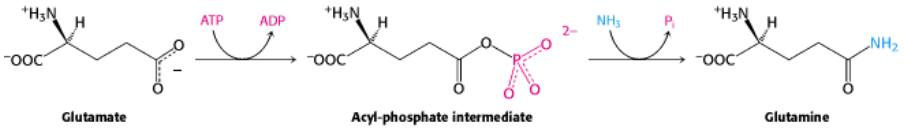
  Glutamate is obtained from the **reductive amination** of **2-oxoglutarate** (or **α-ketoglutarate**). This is catalysed by **glutamate dehydrogenase** (which, interestingly, doesn't distinguish between NADH and NADPH, at least in some species). The reaction involves an intermediate **Schiff base**:



  This reaction is crucial in that it determines the **stereochemistry** of the α carbon atom.

- **Incorporation into glutamine, leading to glutamate [plants and algae]**

  NH_4^+ is incorporated into **glutamate** to give **glutamine**:



This requires the action of **ATP**, which actually takes part in the reaction by forming an **acyl-phosphate intermediate**. The enzyme (**glutamine synthase**) contains a **very high affinity binding site** for **ammonia** (to prevent an **attack** by **water wasting** a molecule of **ATP**).

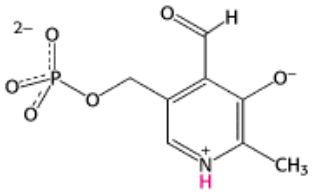
Prokaryotes also contain an **evolutionarily unrelated** enzyme called **glutamate synthase**. Like glutamate dehydrogenase, this catalyses the **reductive amination** of **2-oxoglutarate**, but this time, **glutamine** is the **nitrogen donor**. This results in **two molecules** of **glutamate**, and needs one molecule of **NADPH**. The **side chain** of **glutamine** is **hydrolysed** to generate **ammonia** within the enzyme.

When **NH_4^+** is **limiting**, this is often used to make **glutamate**. Even though this **requires ATP**, it can be more advantageous in limiting NH_4^+ conditions because of the **high affinity to NH_4^+** of **glutamide synthase**. The K_M of glutamate dehydrogenase is rather high for the enzyme to be effective in low NH_4^+ concentrations. Thus, **ATP hydrolysis** is required to **capture ammonia** when it is scarce.

Stage III – Making other amino acids

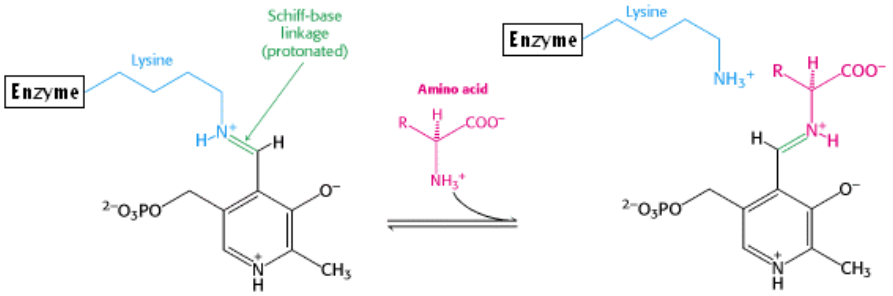
Other amino acids obtain their **amino groups** from **glutamate**, either through **transamination** or **carbon skeleton alteration**.

- **Transaminations** is the **reversible exchange** of an **amino group** between **two keto acids**. Transaminations *from* glutamate are catalysed by **aminotransferases**. This reaction involves a **pyridoxal P** (**vitamin B_6**) **cofactor**:

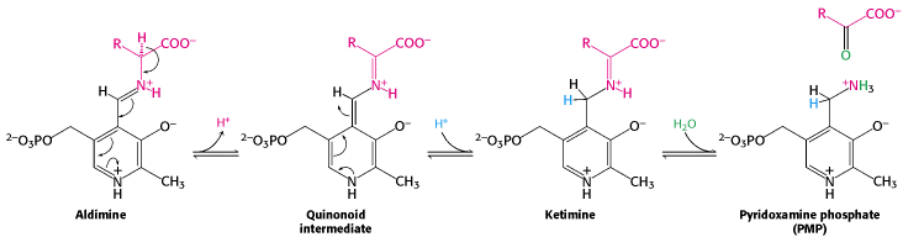


PLP

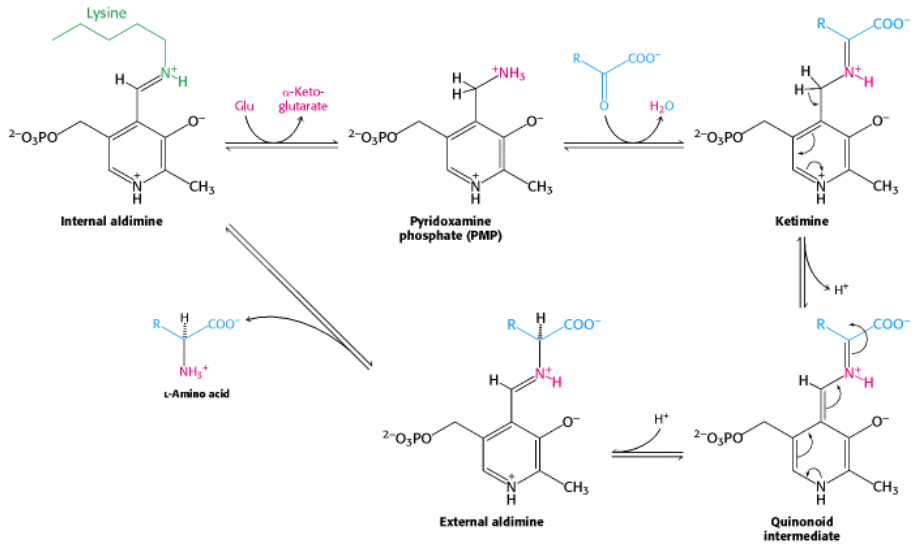
The most important group in this cofactor is the **aldehyde group**, because it forms **Schiff base intermediates** with **amino acid substrates**. Indeed, in the enzyme, the cofactor forms a **Schiff base** with the **amino group** of a **lysine residue**. The enzyme is then **replaced** by the **incoming amino acid**:



PLP then acts as an **electrophilic catalyst**. One of the bonds in the amino acids is **cleaved**, and the resulting **negative charge** is **attracted** to the **positive charge** on the ring nitrogen atom, and thereby **stabilised**.

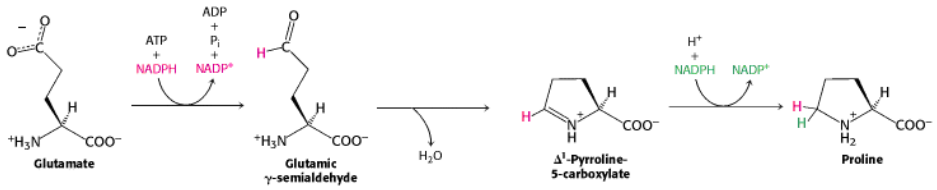


Which bond is cleaved depends on which bond is **perpendicular** to the **π** system. At this point, the **keto acid** now forms a **Schiff base** with **PMP** (but this time, the acid provides the carboxyl group) and is then cleaved:



A **key stage** here is the **protonation** of the **quinonoid intermediate**, because the position the proton is added at determines the **chirality** of the amino acid. An **arginine** residue in the enzyme **orients** the **quinonoid intermediate** so that it is protonated on its **bottom face** and therefore forms the **L-configuration**.

- In some cases, a simple **modification** of the **glutamate molecule** is needed to yield amino acids. For example, **praline**:



Note:

- Humans are not able to make all amino acids – there are 9 **essential amino acids** which humans **cannot synthesise**.
- In general, the **essential amino acids** involve **many steps** in their synthesis, whereas the **nonessential amino acids** involve fewer.
- The exception is **arginine**, because even though its synthesis requires **10 steps**, it can be made from **three steps** from an **intermediate** in the **urea cycle**.
- **Tyrosine** is qualified as **nonessential** because it can be made in **1 step** from **phenylalanine**. If phenylalanine is not present, however, its synthesis requires **10 steps**, and it is an **essential amino acid**.

Obtaining Reducing Power

NADPH is used as the source of **reducing power** for these reactions. The extra **P** group ensures that **NADPH** isn't **re-oxidised** via the **respiratory chain**. In **photosynthetic cells**, this can be obtained straight from the **light reactions** – but what about **non-photosynthetic** cells?

It turns out that there is evidence for **another pathway** of **carbohydrate metabolism** apart from **glycolysis** and the **citric acid cycle**:

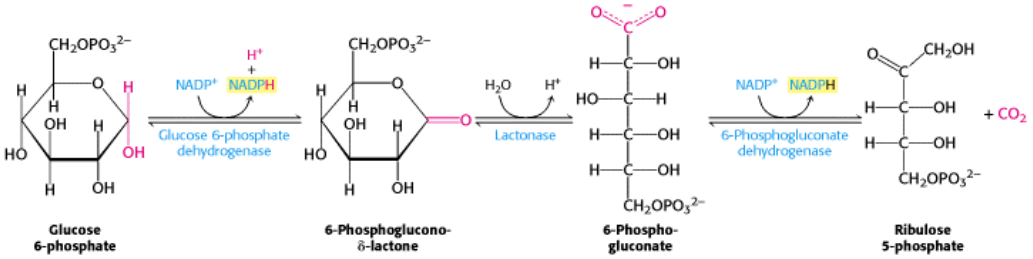
- When **identical samples** of **pea root apices** were supplied with either **[1-^{14}C] glucose** or **[6-^{14}C] glucose**, it is found that in the **former case**, ***more label*** ends up in **CO_2**.
- If glucose is converted to CO_2 *solely* through **glycolysis** and the **citric acid cycle**, though, then the amount of labelled CO_2 in each case should be the **same**, because in the two **triose phosphates** that are produced when **fructose-1,6-bisphosphate** splits, the **carbons** that were **originally** in **positions 1 and 6** in **glucose** occupy **identical positions**.
- These results indicate that there must be **another pathway** in which **glucose's carbon 1** is released early, **without** the release of **carbon 6**. This is called the **oxidative pentose phosphate pathway**.

The **OPPP** has **three purposes**:

- To **generate pentose sugars** for **nucleic acid synthesis**
- It **produces NADPH** for **biosynthesis**.
- It is the **route** for the **metabolic utilisation** of **pentose sugars**.

This pathway consists of two stages – the **oxidative** phase and **non-oxidative** phase:

- The **oxidative section** is as follows:



Glucose 6-phosphate dehydrogenase is in fact **highly specific** for **NADP$^+$** (the K_M for **NAD$^+$** is about 1000 times as great as for NADP$^+$).

- In the **non-oxidative phase**, **ribulose 5-phosphate** is converted into **glyceraldehyde 3-phosphate** and **fructose 6-phosphate** (3 $C_5 \rightarrow$ 2 C_6 + C_3) by **aldolases**, **transketolases** and **other enzymes**. These **carbohydrates** can then be **used for glycolysis** again. This is useful in cells that need **large amounts of NADPH** for **biosynthesis**, but that don't have much need for pentose sugars.

In cells that need *only* **pentose sugars** and **no NADPH**, the **non-oxidative phase** of the **OPPP** can simply be run *backwards*.

These steps are regulated to suit the needs of the cell as follows:

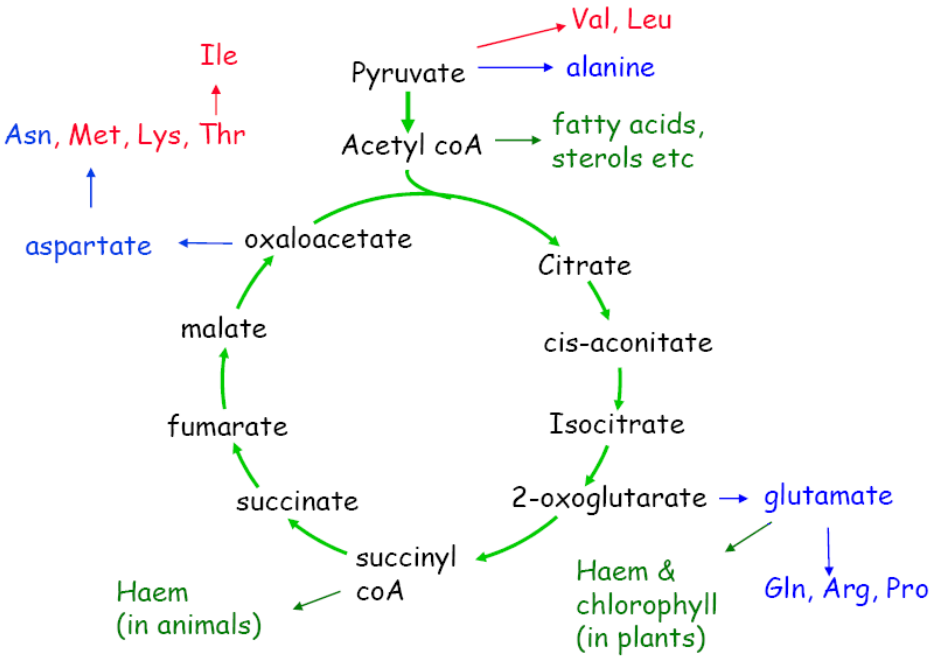
- The **oxidative phase** is regulated by the **presence of NADP$^+$**. ***Low levels*** of **NADP$^+$ inhibit** the **dehydrogenation** of **glucose-6-phosphate**, because NADP$^+$ is needed as the **electron acceptor**. This is intensified by the fact that NADPH **competes** with NADP$^+$ in bindin to the enzyme. This ensures that NADPH is *not* generated unless it is needed.
- The **non-oxidative phase** is controlled primarily by the **availability of substrates**.

The **OPPP** allows **NADPH** to be made **idependently** of the production of **NADH** and **ATP**. In it is active in **growing cells**, but even then, the **flux** is minor – probably **no more** than **20%** of **carbohydrate oxidation**.

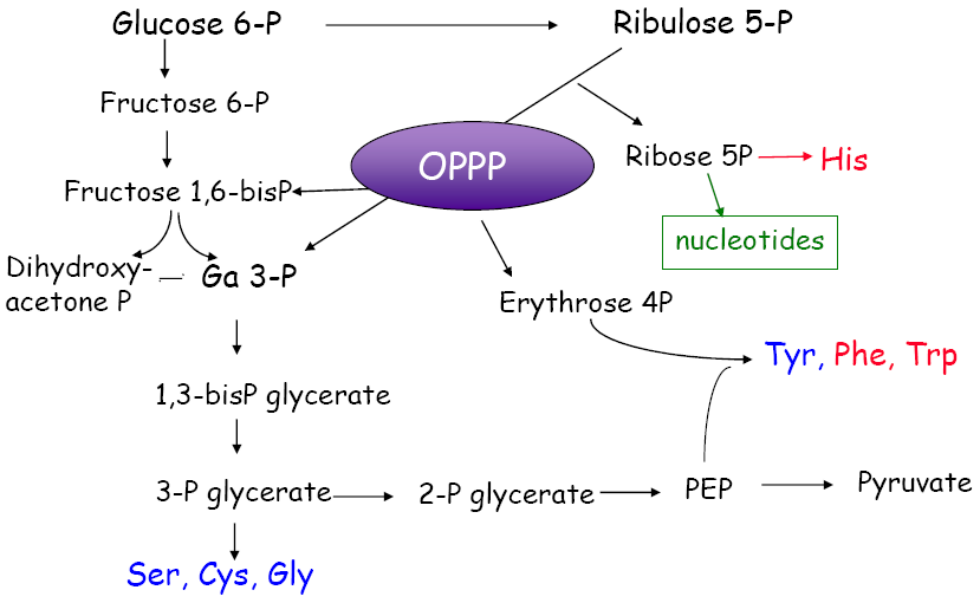
Note, also, that this pathway is *not* a pathway for the **oxidation of glucose**. It does not **oxidise it completely**, or provide ATP. Instead, it **balances** the need of the cell for NADPH or nucleotide synthesis.

Obtaining the Carbon Skeleton

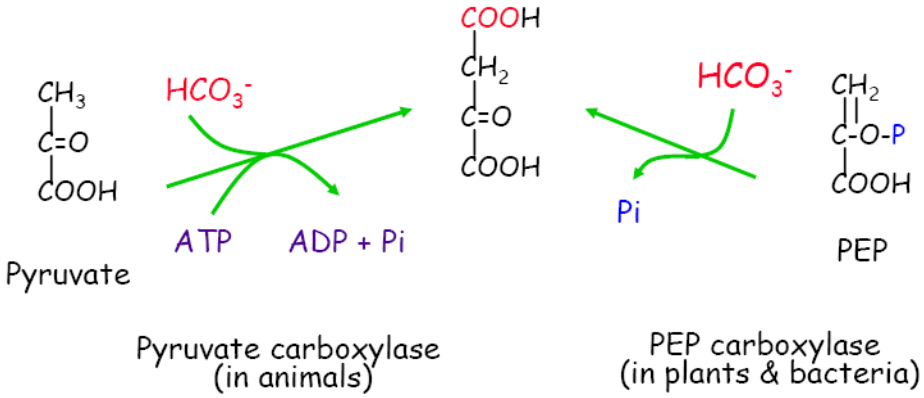
Experiments in which organisms are fed **^{14}C glucose** show that only **30%** of that ^{14}C is released as **CO_2**. The rest is **incorporated** into **biological molecules**. The **carbon skeleton**, therefore, for **most amino acids** comes from **intermediates** of **respiration** (**glycolysis,** the **citric acid cycle** and the **OPPP**)



Up to **50%** of the **carbon** that **enters** the pathway may be used to support biosynthesis.



The cycle, however, is **not anaplerotic** and must be **replenished**. This happens by **carboxylation** of an **intermediate** of **glycolysis** to give **oxaloacetate**. The process is different in plants and animals:



Note that this implies that *all* cells, whether photosynthetic or not, fix CO_2 into organic compounds. There is, however, no **net gain** in carbon, because **re-generating** the **three carbon compound** involves the **loss of CO_2**.

Other Molecules

Note that amino acids are precursors to many other molecules, including lipids, nucleotides, enzyme cofactors, pigments, haemoglobin, etc…