Nitrogen Assimilation

1. Introduction and Overview

Importance of nitrogen to plant metabolism:

- often the limiting nutrient in plants (& agriculture)
- nitrogen can regulates growth processes, due to integration of N and C metabolism

The Nitrogen cycle - position of organic N in the biosphere

- plants can use inorganic nitrogen (N_2, NO_3^-, NH_4^+, but ultimately need in in reduced form (NH_4^+)
- plants have a central position in biosphere N-cycle, compete with microbial **denitrification** and **nitrification**
- **nitrate reduction** is different from **nitrogen fixation**

Overview of reactions

(Uptake ->) **NO_3^-** -> **NO_2^-** -> **NH_4^+**-> **glutamine** -> glutamate -> amino acids

Uptake, **reduction, assimilation, biosynthesis**

*Note: **Nitrogen Fixation** is different (N_2-> NH_4^+-> glutamine -> amino acids) (occurs only in some plants)*

Topics:
1. NO_3^- uptake & reduction (to NH_4^+)
2. NH_4^+ assimilation (get to organic N) -> glutamate
3. principles of synthesis of amino acids
4. nitrogen fixation as a special case (legumes)

2. Uptake and Reduction of NO_3^- : NO_3^- uptake is very dynamic (microbial competition)

- NO_3^- concentration in soil is highly variable [10µM - 100mM]
- specific transporters with **saturable kinetics** ($2H^+$ / NO_3^- co-transporter mechanism)
  i. LATS (low-affinity transport system, (non-)saturable)
  ii. HATS (high-affinity transport system, *saturable*)

=> two systems for both high and low NO_3^- levels

` - uptake activity inducible by NO_3^- (= adaptive response)

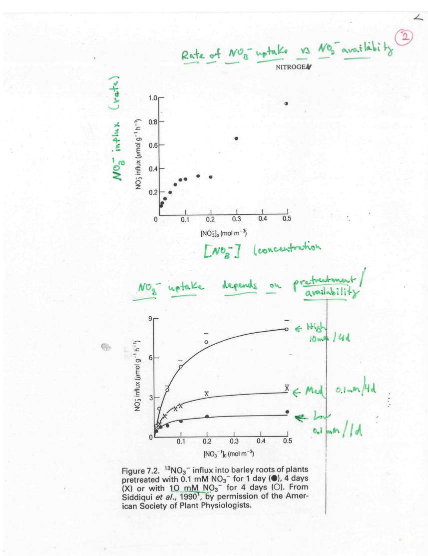
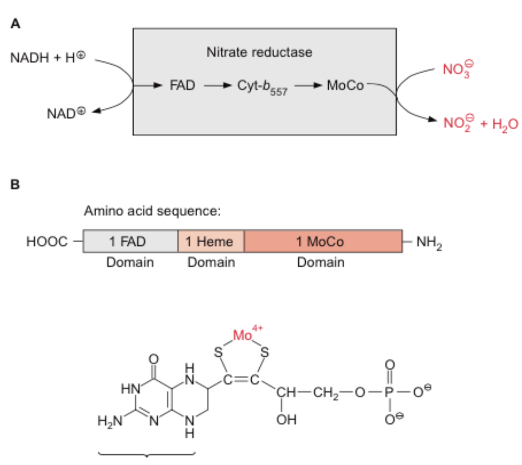


Figure 7.2. $^{13}NO_3^-$ influx into barley roots of plants pretreated with 0.1 mM NO_3^- for 1 day (●), 4 days (X) or with 10 mM NO_3^- for 4 days (○). From Siddiqui *et al.*, 1990[1], by permission of the American Society of Plant Physiologists.



Reduction of NO_3^- to NH_4^+ in two steps (8 electrons)

- N oxidation number from +5 to -3 = 8 e^-(requires 2 enzymes)
- requires a lot of **reducing power** (NADH, NADPH or **ferredoxin**)
- occurs in leaves or roots (the latter especially in trees)

Step 1: *Enzyme:* **Nitrate reductase (NR)**

- Net reaction: $NO_3^- + 2 e^- + 2 H^+$ ----> $NO_2^- + H_20$
- enzyme has three several **domains** (= structural units)
- **prosthetic groups** and **co-factors:** FAD, haem, **Mo^{4+}**
- electrons come from **NADH** (or **NADPH**)
- cytoplasmic localization
- ClO_3^- (chlorate) is also reduced if it is present. The product, chlorite, is toxic.

Step 2: *Enzyme:* **Nitrite reductase (NiR)**

- Net reaction: $NO_2^- + 6 e^- + 8 H^+$ ----> $NH_4^+ + 2 H_2O$
- prosthetic groups: iron-sulfur centers (4Fe-4S), FAD,sirohaem (also contains Fe)
- electrons source is **ferredoxin** (or NADPH);
- located in chloroplast/plastid
- NO_2^- is toxic (thus, NiR capacity needs to be very high to use it up)

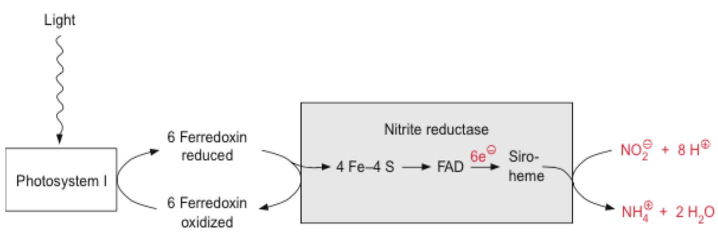


Figure 10.4 Nitrite reductase in chloroplasts transfers electrons from ferredoxin to nitrite. Reduction of ferredoxin by photosystem I is shown in Figure 3.16.

Regulation of NO_3^- reduction is tight (due to toxicity, and coordination of N to C)

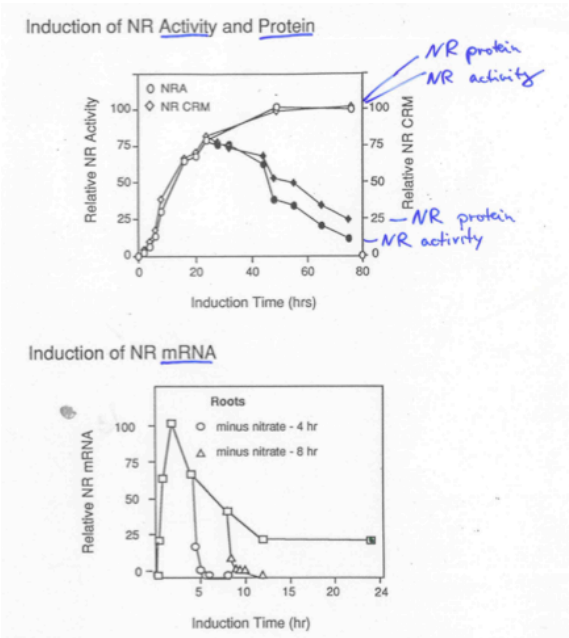
i. transcriptional regulation of NR (also NiR, HATS)

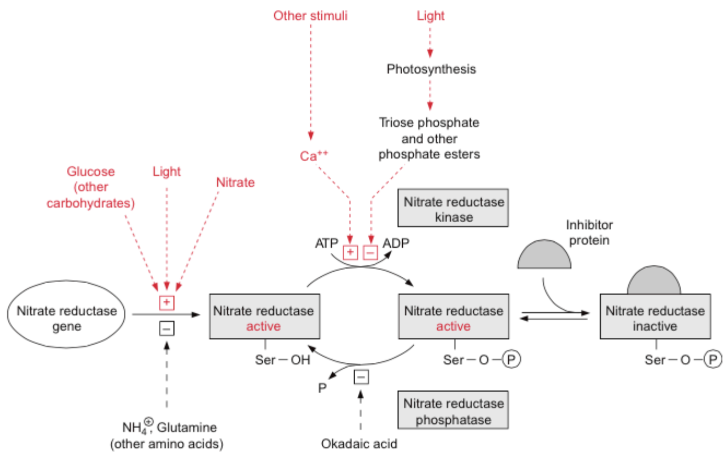
inducers: NO_3^-, light, sucrose, circadian cycle

repressors: NH_4^+, glutamine

ii. tight post-transcriptional regulation of NR:

- rapid NR mRNA and protein turnover (short half-life)
- phosphorylation and inactivation are sequential





3. NH_4^+ assimilation to glutamic acid (glutamate) occurs in two linked steps

Step 1: ***Glutamine synthetase***

glutamate + NH_4^+ + ATP -> glutamine + ADP + Pi

Step 2: **Glutamate synthase**

glutamine + α-ketoglutarate + ferredoxin (reduced) -->
2x glutamate + 2x ferredoxin (oxidized)

- source of electrons is
- closely coupled to glutamine synthetase
- chloroplastic, but there are cytosolic forms for N fixers

Regulation of both enzymes:

- transcription is activated by NO_3^-, NH_4^+, light (both enzymes)
- large amount present - rapid assimilation of NH_4^+ (toxic)
- connected to **photorespiration** (= *metabolic reactions to recover 2-P-glycollate (side reaction to carbon fixation*)

Herbicides frequently target N-metabolism (glufosinate: ('Basta', 'Liberty') is a glutamate analog

Some differences in assimilation in the leucoplast vs chloroplast

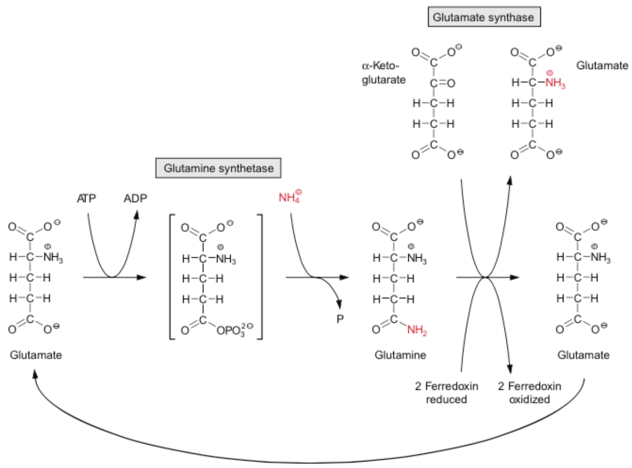
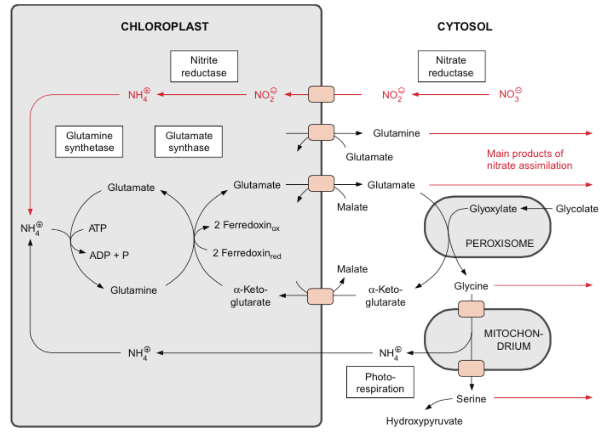


Figure 7.9 Sequence of reactions of the fixation of ammonia with subsequent synthesis of glutamate from α-ketoglutarate.



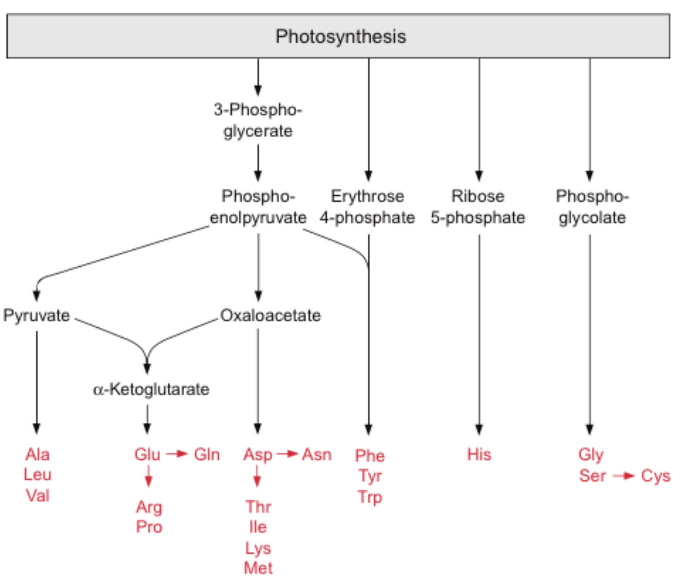
4. Synthesis of amino acids and other nitrogenous metabolites

- Amino Acid synthesis: via Gln/Glu, organized by C-skeleton

- *Aminotransferase* enzymes are key to N metabolism
  - they transfer amino group from glutamate (donor) to carbon skeleton --> produce a new amino acid

  (Eg) aspartate aminotransferase
  glutamate + oxaloacetate <---> a-ketoglutarate + aspartate

  (Eg) alanine aminotransferase
  glutamate + pyruvate <---> a-ketoglutarate + alanine



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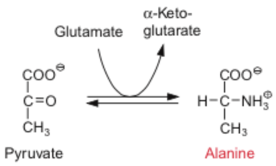

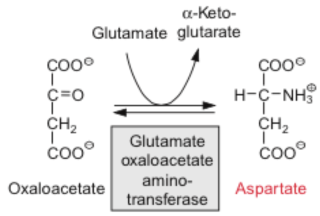

Glutamate has a **central position in N metabolism** and is used a nitrogen donor for many molecules:

---> all other amino acids (in families - often late in the pathway)
---> nitrogenous bases (nucleic acids)
---> chlorophyll (glu)
---> alkaloids (from trp, tyr)

Overall transport of nitrogen in plants

- whole plant perspective: nitrate reduction in leaves or roots
- transport of glutamine, asparagine or alanine
- ureides: allantoate and allantoic acid are alternate transport forms of N (legume plants)