Nitrate & Ammonia Assimilation

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Nitrogen Assimilation

- Although nitrogen gas is abundant in the atmosphere, few microorganisms can reduce the gas and use it as a nitrogen source.
- Most must incorporate either ammonia or nitrate.

Ammonia Incorporation

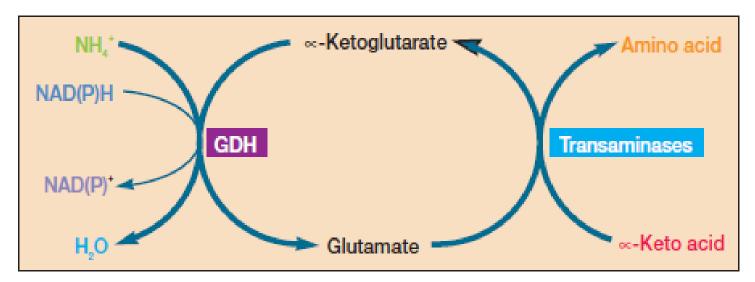
- Ammonia nitrogen can be incorporated into organic material relatively easily and directly because it is more reduced than other forms of inorganic nitrogen.
- Some microorganisms form the amino acid alanine in a reductive amination reaction catalyzed by **alanine dehydrogenase**.

Pyruvate + NH_4^+ + NADH (NADPH) + H^+ \rightleftharpoons L-alanine + NAD^+ (NADP⁺) + H_2O

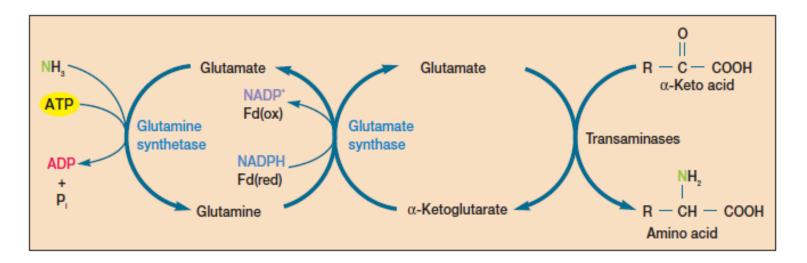
- The major route for ammonia incorporation often is the formation of glutamate from α -ketoglutarate (a TCA cycle intermediate).
- Many bacteria and fungi employ **glutamate dehydrogenase**, at least when the ammonia concentration is high.

 $\alpha \text{-ketoglutarate} + \text{NH}_{4}^{+} + \text{NADPH}(\text{NADH}) + \text{H}_{4}^{+}$ $\rightleftharpoons \text{glutamate} + \text{NADP}^{+}(\text{NAD}^{+}) + \text{H}_{2}\text{O}$

- Different species vary in their ability to use NADPH and NADH as the reducing agent in glutamate synthesis.
- Once either alanine or glutamate has been synthesized, the newly formed α-amino group can be transferred to other carbon skeletons by transamination reactions to form aminoacids.
- Microorganisms have a number of transaminases, each of which catalyzes the formation of several amino acids using the same amino acid as an amino group donor.
- When glutamate dehydrogenase works in cooperation with transaminases, ammonia can be incorporated into a variety of amino acids.

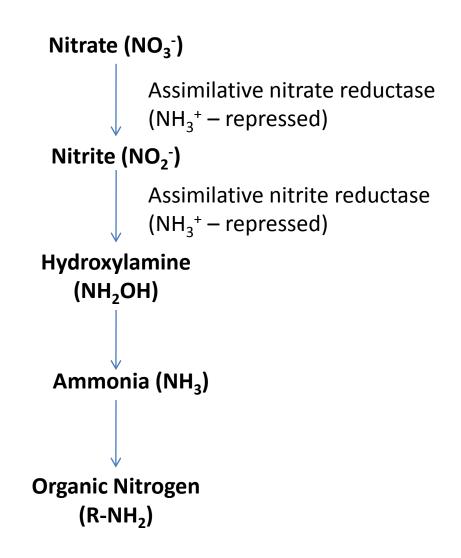


- A second route of ammonia incorporation involves two enzymes acting in sequence, glutamine synthetase and glutamate synthase.
- Ammonia is used to synthesize glutamine from glutamate, then the amide nitrogen of glutamine is transferred to α -ketoglutarate to generate a new glutamate molecule.
- Because glutamate acts as an amino donor in transaminase reactions, ammonia may be used to synthesize all common amino acids when suitable transaminases are present.
- Both ATP and a source of electrons, such as NADPH or reduced ferredoxin, are required.
- This route is present in *Escherichia coli, Bacillus megaterium,* and other bacteria.
- The two enzymes acting in sequence operate very effectively at low ammonia concentrations, unlike the glutamate dehydrogenase pathway.



Nitrate Assimilation

- The nitrogen in nitrate (NO_3^-) is much more oxidized than that in ammonia.
- Nitrate must first be reduced to ammonia before the nitrogen can be converted to an organic form.
- This reduction of nitrate is called **assimilatory nitrate reduction**, which is not the same as that occurring during anaerobic respiration and dissimilatory nitrate reduction
- In assimilatory nitrate reduction, nitrate is incorporated into organic material and does not participate in energy generation.
- However, in dissimilative nitrate reduction nitrate is used as an alternative electron acceptor in energy generation.
- Assimilatory nitrate reduction takes place in the cytoplasm in bacteria.
- The first step in nitrate assimilation is its reduction to nitrite by nitrate reductase, an enzyme that contains both FAD and molybdenum .
- Nitrite is next reduced to ammonia with a series of two electron additions catalyzed by nitrite reductase and possibly other enzymes. Hydroxylamine may be an intermediate.
- The ammonia is then incorporated into amino acids by the routes already described.



- Assimilative nitrate reductase (Molybdenum containing) is a cytoplasmic enzyme.
- Assimilative nitrate reduction can occur quite well under fully aerobic condition.
- Pathway found in plants, fungi, bacteria.

Questions

- Write an essay on assimilation of nitrogen
 Ans. Write both ammonia and nitrate assimilation
- Explain ammonia assimilation.
- Write a note on nitrate assimilation.
 Ans. Write conversion of nitrate to ammonia, then ammonia assimilation
- Differentiate between assimilative and dissimilative nitrate reduction.