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NITROGEN METABOLISM

All the living organisms are basically composed of carbon, hydrogen, oxygen, nitrogen and many other forms of chemical elements. These elements contribute to finally organize various biomolecules present in a cell. Nitrogen is next to carbon in importance in living organisms. In a living cell, nitrogen is an important constituent of amino acids, proteins, enzymes, vitamins, alkaloids and some growth hormones. Therefore, study of nitrogen metabolism is absolutely essential because the entire life process is dependent on these nitrogen-containing molecules. In this lesson, you will learn about various aspects of nitrogen metabolism including nitrogen fixation and nitrogen assimilation in plants.

OBJECTIVES

After completing this lesson, you will be able to:

- *describe the modes of nitrogen fixation (both biological and abiological);*
- *explain the steps involved in nitrogen fixation by free living organisms;*
- *explain the mode of symbiotic nitrogen fixation in leguminous plants;*
- *describe the assimilation of nitrate and ammonia by plants;*
- *describe amino acid synthesis in plants.*

10.1 MOLECULAR NITROGEN

Nitrogen is primarily present in the atmosphere freely as dinitrogen or nitrogen gas. It is present in the combined form as Chile saltpetre or sodium nitrate and Chile in South America is the major source of this nitrate nitrogen.

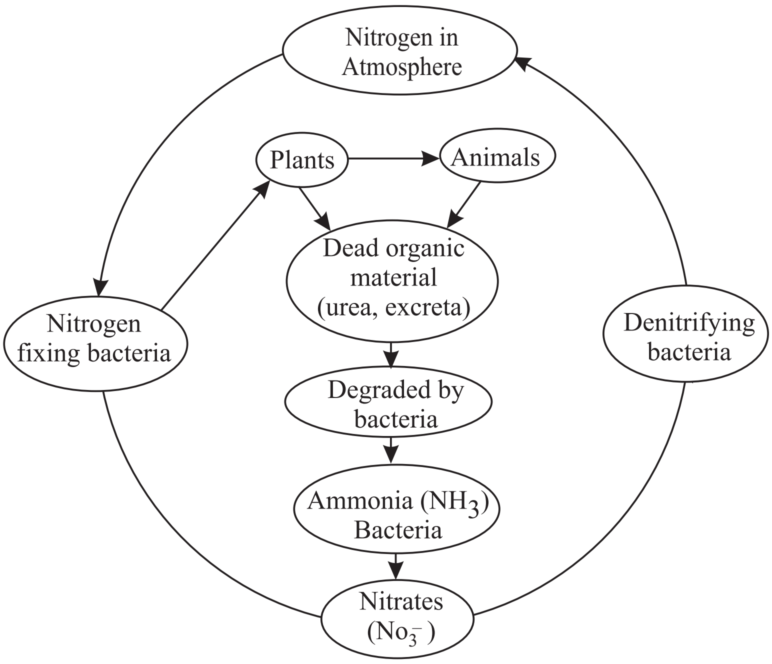
Molecular Nitrogen or diatomic nitrogen (N_2) is highly stable as it is triple bonded ($N\equiv N$). Because of this stability, molecular nitrogen as such is not very reactive in the atmosphere under normal conditions. In the atmosphere molecular nitrogen is 78.03% by volume and it has a very low boiling point (-195.8°C) which is even lower than that of oxygen. Proteins present in living organisms contain about 16% nitrogen.

10.1.1 Nitrogen Cycle

Nitrogen is an essential constituent of living beings. Nitrogenous bases are part of nucleic acids and proteins are made up of amino acids of which Nitrogen is an important consituent. You already know about the importance of these two biomolecules.

Air has 78% N_2 but most of the living beings cannot utilize this atmospheric Nitrogen. Nitrogen cycle converts this nitrogen into a usable form. Lightning fixes Nitrogen to NH_3, and nitrogen fixing bacteria like *Rhizobium* (which live in roots of leguminous plants like pea, rajma, beans, pulses etc.) also convert N_2 into NH_3. Most plants absorb nitrates from soil and reduce it to NH_3 in the cells for further metabolic reactions. Dead organisms and their excreta like urea are decomposed by bacteria into NH_3 and by a different set of bacteria into nitrates. These are left in the soil for use by plants. In this way Nitrogen cycle is self regulated but human activities have caused steady loss of soil Nitrogen.

NITROGEN CYCLE



INTEXT QUESTIONS 10.1

1. What is the percent by volume of nitrogen gas in the atmosphere?

   ....

2. Name two biomolecules that contain nitrogen in plants.

   ....

3. Why nitrogen is a stable molecule?

   ....

4. What is the percentage of nitrogen in protein?

   ....

5. What is the boiling point of nitrogen?

   ....

6. Choose the correct option:

   Nitrogen fixation is the conversion of :

   (a) atmospheric Nitrogen $\xrightarrow{\text{into}}$ Nitrates

   (b) atmospheric Nitrogen $\xrightarrow{\text{into}}$ Ammonia

   (c) atmospheric Nitrogen $\xrightarrow{\text{into}}$ Amino acids

   (d) both (a) and (b)

7. Nitrogen content of biosphere remains constant because of :

   (a) Nitrogen cycle

   (b) Nitrogen fixation

   (c) Industrial pollution

   (d) Absorption of nitrogen

8. Nitrates are converted into nitrogen by microbes called ....

10.2 NITROGEN FIXATION (BIOLOGICAL AND ABIOLOGICAL)

The conversion of molecular nitrogen into compounds of nitrogen especially ammonia is called **nitrogen fixation**. Nitrogen fixation, is a reductive process i.e., nitrogen fixation will stop if there is no reducing condition or if oxygen is present. This nitrogen fixation may take place by two different methods – abiological and biological.

10.2.1 Abiological nitrogen fixation

In abiological nitrogen fixation the nitrogen is reduced to ammonia without involving any living cell. Abiological fixation can be of two types : industrial and natural. For example, in the Haber's process, synthetic ammonia is produced by passing a mixture of nitrogen and hydrogen through a bed of catalyst (iron oxides) at a very high temperature and pressure.

$$N_2 + 3H_2 \xrightarrow[\text{1000 atmosphere}]{500°C} 2NH_3$$

This is industrial fixation wherein nitrogen gets reduced to ammonia.

In natural process nitrogen can be fixed especially during electrical discharges in the atmosphere. It may occur during lightning storms when nitrogen in the atmosphere can combine with oxygen to form oxides of nitrogen

$$N_2 + O_2 \longrightarrow 2NO \xrightarrow{O_2} 2NO_2$$

These oxides of nitrogen may be hydrated and trickle down to earth as combined nitrite and nitrate.

10.2.2 Biological nitrogen fixation

Chemically, this process is same as abiological. Biological nitrogen fixation is reduction of molecular nitrogen to ammonia by a living cell in the presence of enzymes called nitrogenases.

INTEXT QUESTIONS 10.2

1. Define nitrogen fixation.

   ....

2. Which industrial process is utilized for converting nitrogen to ammonia?

   ....

3. Distinguish between biological and abiological nitrogen fixation.

   ....

4. Name the enzyme that helps in nitrogen fixation in living cells.

   ....

5. Which gas prevents nitrogen fixation?

   ....

10.3 NITROGEN FIXATION BY FREE LIVING ORGANISMS AND SYMBIOTIC NITROGEN FIXATION

Nitrogen fixation is a distinctive property possessed by a select group of organisms, because of the presence of the enzyme nitrogenase in them.

The process of nitrogen fixation is primarily confined to microbial cells like bacteria and cyanobacteria. These microorganisms may be independent and free living (Table 10.1).

Table 10.1 : Some free living microbes which fix nitrogen

Organisms	Status
Clostridium	Anaerobic bacteria (Non-photosynthetic)
Klebsiella	Facultative bacteria (Non-photosynthetic)
Azotobacter	Aerobic bacteria (Non-photosynthetic)
Rhodospirillum	Purple, non-sulphur bacteria (Photosynthetic)
Anabaena	Cyanobacteria (Photosynthetic)

Some microbes may become associated with other oragnisms and fix nitrogen. The host organism may be a lower plant or higher plant. The host organism and the nitrogen fixing microbes establish a special relationship called **symbiosis** and this results in symbiotic nitrogen fixation (Table 10.2).

Table 10.2 : Some symbiotic nitrogen fixing organisms

System	Symbionts
Lichens	Cyanobacteria and Fungus.
Bryophyte	Cyanobacteria and *Anthoceros*.
Pteridophyte	Cyanobacteria and *Azolla*.
Gymnosperm	Cyanobacteria and *Cycas*.
Angiosperms	Legumes and *Rhizobium*.
Angiosperms	Non leguminous plants and actinomycete (Such as *Alnus*, *Myrica*, *Purshia*).
Angiosperm	Brazilian grass (*Digitaria*), Corn and *Azospirillum*.

10.3.1 Mechanism of Biological Fixation of Nitrogen

Nitrogen fixation requires

(i) the molecular nitrogen

(ii) a strong reducing power to reduce nitrogen like reduced FAD (Flavin adenine dinucleotide) and reduced NAD (Nicotinamide Adenine Dinucleotide)

(iii) a source of energy (ATP) to transfer hydrogen atoms from $NADH_2$ or $FADH_2$ to dinitrogen and

(iv) enzyme nitrogenase

(v) compound for trapping the ammonia formed since it is toxic to cells.

The reducing agent ($NADH_2$ and $FADH_2$) and ATP are provided by photosynthesis and respiration.

The overall **biochemical process** involves stepwise reduction of nitrogen to ammonia. The enzyme nitrogenase is a Mo-Fe containing protein and binds with molecule of nitrogen (N_2) at its binding site. This molecule of nitrogen is then acted upon by hydrogen (from the reduced coenzymes) and reduced in a stepwise manner. It first produces diamide (N_2H_2) then hydrazime (N_2H_4) and finally ammonia ($2NH_3$).

NH_3 is not liberated by the nitrogen fixers. It is toxic to the cells and therefore these fixers combine NH_3 with organic acids in the cell and form amino acids.

The general equation for nitrogen fixation may be described as follows:

$$N_2 + 16ATP + 8H^+ + 8e^- \longrightarrow 2NH_3 + 16ADP + 16Pi +$$

> Molecular nitrogen is a very stable molecule. Therefore, sufficient amount of cell energy in the form of ATP is required for stepwise reduction of nitrogen to ammonia.

In legumes, nitrogen fixation occurs in specialized bodies called **root nodules**. The nodules develop due to interaction between the bacteria *Rhizobium* and the legume roots (see diagram 6.4c). The biochemical steps for nitrogen fixation are same. However, legume nodules possess special protein called LEGHEMOGLOBIN. The synthesis of leghemoglobin is the result of symbiosis because neither bacteria alone nor legume plant alone possess the protein. Recently it has been shown that a number of host genes are involved to achieve this. In addition to leghemoglobin, a group of proteins called **nodulins** are also synthesized which help in establishing symbiosis and maintaining nodule functioning.

> Leghemoglobin is produced as a result of interaction between the bacterium and legume roots. Apparently, Rhizobium gene codes for Heme part and legume root cell gene codes for Globin moiety. Both the coded products together consitute the final protein leghemoglobin. During N_2-fixaion, function of Leghemoglobin is to act as Oxygen-scavenger so that the enzymes, Nitrogenages then, convert N_2 to NH_3 under anaerobic condition.

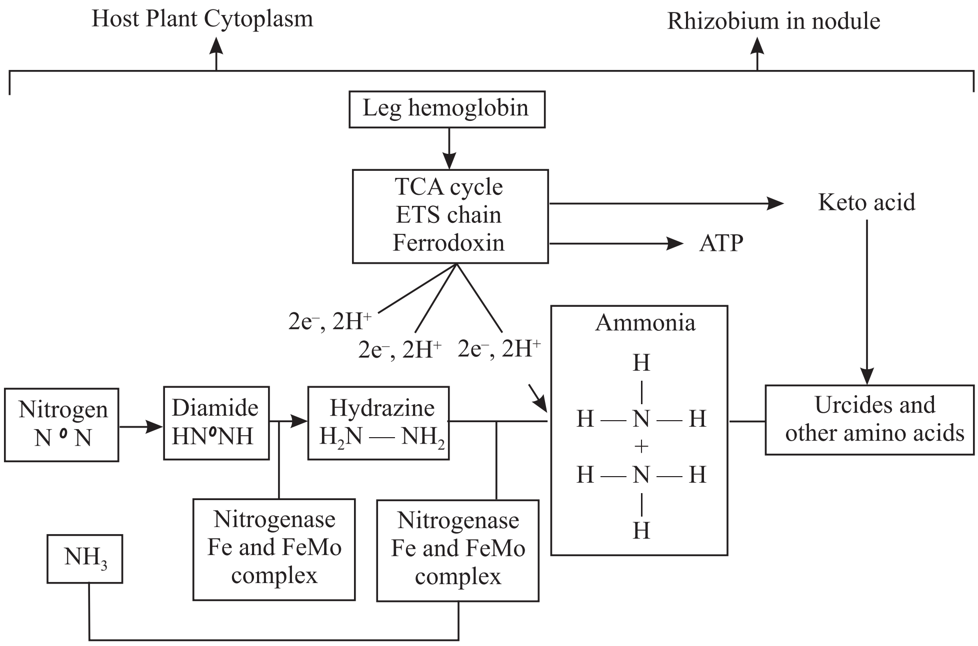


Fig. 10.1 Simplified flowsheet of biochemical steps for nitrogen fixation

Leghemoglobin is considered to lower down the partial pressure of oxygen and helps in nitrogen fixation. However, this function is specific for legumes only because free living microbes do not possess nitrogen fixing leghemoglobin. Moreover, it has also not been found in cyanobacterial symbiosis with other plants, which fix N_2 under aerobic condition.

INTEXT QUESTIONS 10.3

1. Match the following:

	A		B
(i)	*Azotobacter*	(a)	anaerobic nitrogen fixer.
(ii)	*Clostridium*	(b)	aerobic nitrogen fixer
(iii)	*Lichens*	(c)	aerobic nitrogen fixing cyanobacterium
(iv)	*Anabaena*	(d)	symbiotic nitrogen fixer.

2. Which Gymnospermous plant fixes nitrogen?

   ....

3. Is there any other gas evovled during nitrogen fixation? If yes, name the gas evolved.

   ....

4. How many ATP molecules are required to reduce a single molecule of nitrogen?

   ....

5. What is the major source of electrons for reduction of nitrogen?

   ....

6. Match the following:

	A		B
(i)	Leghemoglobin	(a)	cyanobacterium
(ii)	*Anabaena*	(b)	Legumes
(iii)	Reductive process	(c)	nitrogen fixation

7. Name the proteins that help in establishing symbiosis and maintain root nodule functioning in legumes.

   ....

10.4 NITRATE AND AMMONIA ASSIMILATION BY PLANTS

As pointed out in the previous section, nitrogen fixation is confined to selected microbes and plants. But all plants require nitrogen because it has a role to play in the general metabolism. Therefore, plants which do not fix nitrogen, use other combined nitrogen sources such as nitrate and ammonia for carrying on metabolic activity.

Nitrate is absorbed by most plants and reduced to ammonia with the help of two different enzymes. The first step conversion of nitrate to nitrite is catalyzed by an enzyme called nitrate reductase. This enzyme has several other important constituents including FAD, cytochrome, NADPH or NADH and molybdenum.

$$NO_3^- + NADH + H^+ \xrightarrow{\text{Nitrate reductase}} NO_2^- + NAD^+ + H_2O$$

The overall process of nitrate reduction take place in the cytosol and is an energy dependent reaction.

The enzyme nitrate reductase has been studied in many plants and it is observed that the enzyme is continuously synthesized and degraded. The enzyme nitrate reductase is inducible. This means that increase in nitrate concentration in the cytosol induces more of nitrate redutase to be synthesized. However, when excess NH_4^+ is produced then it has a negative effect on the synthesis of nitrate reductase. In plants, it has also been observed that light also increases nitrate reductase when nitrate is available.

In the second step the nitrite so formed is further reduced to ammonia and this is catalyzed by the enzyme nitrite reductase. Nitrite present in the cytosol is transported into chloroplast or plastids where it is reduced to ammonia.

$$NO_2^- + 3NADPH + 3H^+ \xrightarrow{\text{Nitrite reductase}} NH_3 + 3NADP^+$$

The enzyme nitrite reductase is able to accept electrons from sources such as NADH, NADPH or $FADH_2$. Besides, reduced ferredoxin has also been shown to provide electrons to nitrite reductase for reducing nitrite to ammonia. Ammonia so formed has to be utilized quickly by plants because accumulation of ammonia has a toxic effect. Some plants including algae leach out excess ammonia which can further be oxidized to nitrite and nitrate by microorganisms in the soil or water.

INTEXT QUESTIONS 10.4

1. Which is the most reduced form of inorganic nitrogen?

   ....

2. Match the following:

	A		B
(*i*)	Nitrate reductase	(a)	nitrogen fixation
(*ii*)	Nitrite reductase	b)	nitrate reduction
(*iii*)	Nitrogenase	c)	nitrite reduction

3. In which part of the cell, reduction of nitrate to nitrite occurs?

   ....

4 Which is the most oxidized form of inorganic nitrogen?

   ....

5 In which plant organelle reduction of nitrite to ammonia is catlyzed by the enzyme nitrite reductase?

   ....

10.5 AMINO ACID SYNTHESIS BY PLANTS

As you have noticed that ammonia formation is achieved by plants either by (i.) nitrogen fixation or (ii) by reduction of nitrate to nitrite. Ammonium (NH_4^+) is the most reduced form of inorganic combined nitrogen. This ammonium now becomes the major source for the production of amino acids, which are the building blocks of enzymes and proteins. Amino acids have two important chemical groups. (i) amino group (NH_2) and (ii) carboxy1 group (-COOH).

$$\begin{array}{c} \mathrm{H} \\ | \\ \mathrm{R} - \mathrm{C} - \mathrm{COOH} \\ | \\ \mathrm{NH_2} \end{array}$$

Fig. 10.2 A typical amino acid with functional groups. R represents alkyl group.

Ammonium so produced is the major source of amino group. However, the carboxyl group has to be provided by other organic molecule synthesized by the plants. There are two major reactions for amino acid biosynthesis in plants:

10.5.1 Reductive amination reaction:

In this reaction, ammonia combines with a keto acid. The most important keto acid is the alpha ketoglutaric acid produced during the operation of Krebs cycle (see lesson 12 Plant Respiration). The keto acid then undergoes enzymatic reductive amination to produce an amino acid.

$$\underset{\text{(keto acid)}}{\alpha\text{-ketoglutaric acid}} + NH_3 \xrightarrow{\text{glutamate dehydrogenase}} \underset{\text{(amino acid)}}{\text{Glutamic}}$$

Similarly another amino acid called aspartic acid is produced by reductive amination of oxaloacetic acid.

> It has been noted that reductive amination respresents the major 'port of entry' for ammonia into the metabolic stream in plants. This initiates synthesis of glutamic acid followed by other amino acids.

10.5.2 Transamination reaction

This is another very important reaction for amino acid biosynthesis. The reaction involves transfer of amino group, from already synthesized amino acid, to the keto acid.

$$\underset{\text{(Keto acid)}}{\alpha\text{-Ketoglutaric acid}} + \underset{\text{(Amino acid)}}{\text{Aspartic acid}} \xrightarrow{\text{Transaminase}} \underset{\text{(Amino acid)}}{\text{Glutamic acid}} + \underset{\text{(Keto acid)}}{\text{Oxaloacetic acid}}$$

In the above reaction, aspartic acid has transferred its amino group (NH_2) to the α-ketoglutaric acid to synthesize glutamic acid and release keto acid. The reaction is catalyzed by enzymes called **transaminases**. A large number of amino acids are synthesized by this transamination reaction. Amino acids are organic molecules containing nitorgen. The incorporation of amino group, from ammonium, into keto acids represents the major step for synthesis of nitrogenous organic biomolecules.

Notes

INTEXT QUESTIONS 10.5

1. Match the following:

	A	B
(i)	Amino acid	(a) keto acid
(ii)	Glutamic acid	(b) amino group and carboxyl group
(iii)	α-ketoglutaric acid	(c) amino acid

2. Name two biochemical reactions for biosynthesis of amino acids in plants.

   ....

3. Which group of enzymes catalyzes transamination reaction?

   ....

4. What is the source of amino group for amino acid synthesis in reductive amination reaction?

   ....

5. Which keto acid is the source for synthesis of glutamic acid?

   ....

WHAT YOU HAVE LEARNT

- Nitrogen is an important constituent of several biomolecules such as amino acids, proteins and enzymes.
- Molecules such as vitamins, alkaloids, nucleic acids, pigments and some growth hormones also contain nitrogen.
- Molecular nitrogen is triple bonded and stable.
- Nitrogen fixation is the reduction of nitrogen to ammonia.
- Abiological nitrogen fixation is an industrial process (Haber's process)
- Biological nitrogen fixation takes place in a living cell.
- The enzyme that catalyzes nitrogen fixation is Nitrogenase.
- Nitrogen fixation may take place in free living organisms or in symbiotic systems.
- There are many symbiotic nitrogen fixation systems such as Lichens, Pteridophytes, Bryophytes, Gymnosperms and Legumes.
- Cyanobacteria is the symbiotic component in Lichens, Bryophytes, Pteridophytes and Gymnosperms.
- In Legumes, the symbiont is a species of bacterium *Rhizobium*.
- Source of electrons and energy for nitrogen fixation is generally pyruvic acid after it enters Krebs' cycle during cell-respiration.
- Hydrogen gas evolution may also accompany nitrogen fixation process.

- Nitrate is the most oxidized form and ammonium is the most reduced form of nitrogen.
- Nitrate is reduced to nitrite by an enzyme nitrate reductase.
- Amino acids have two functional groups, namely, amino group and carboxyl group.
- Amino acids may be produced by reductive amination of keto acids.
- Amino acids may be produced by transamination reaction.
- Reductive amination reactions are catalyzed by dehydrogenases.
- Transamination reactions are catalyzed by transaminases.

TERMINAL EXERCISES

1. Define nitrogen fixation.
2. Which form of combined nitrogen may be formed during lightening storms?
3. Name three biomolecules other than enzymes and proteins, which contain nitrogen.
4. Name one aerobic and one anaerobic bacterium, which fixes nitrogen.
5. Which amino acid is synthesized due to reductive amination of α-ketoglutaric acid?
6. Differentiate between biological and abiological nitrogen fixation.
7. What is required for biological nitrogen fixation?
8. How does human hemoglobin differ from leghemoglobin?
9. What is the function of leghemoglobin?
10. What are the functional differences between nitrate reductase and nitrite reductase?
11. What is the difference between nitrogen fixation and nitrogen assimilation? Describe in brief the process of abiological nitrogen fixation.
12. Describe in brief various steps involved in biological nitrogen fixation.
13. Enumerate various free living and symbiotic nitrogen fixing systems with suitable examples.
14. What are the major differences between free living and leguminous nitrogen fixing organisms?
15. Describe in brief nitrate and nitrite reduction in plants..
17. Describe in brief the reductive amination reactions for synthesis of amino acids in plants.
18. Describe the transamination reaction for synthesis of amino acids in plants. How does this differ from reductive amination?

ANSWERS TO INTEXT QUESTIONS

10.1 1. 78.03 percent 2. proteins and enzymes.
3. Because it is triple bonded. 4. 16 percent.
5. –195.8°C. 6. (b)
7. (a) 8. Denitrifying Bacteria

10.2 1. Conversion of molecular nitrogen to ammonia.
2. Haber's process.
3. Biological nitrogen fixation takes place in a living cell and abiological fixation without a living cell.
4. Nitrogenase. 5. Oxygen.

10.3 1. (i) b (ii) a (iii) d (iv) c
2. Cycas. 3. Yes, Hydrogen gas.
4. 16 ATP 5. Reduced coenzymes such as Ferrodoxin
6. (i) b (ii) a (iii) c 7. Nodulins.

10.4 1. NH_4
2. (i) b (ii) c (iii) a
3. Cytosol. 4. Nitrate. 5. Chloroplast.

10.5 1. (i) b (ii) c (iii) a
2. Reductive amination and transamination.
3. Transaminases. 4. Ammonia.
5. Alpha ketoglutaric acid.