LEARNING OBJECTIVES

- Distinguish between component I and II of the nitrogenase enzyme and its role in biological nitrogen fixation

Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. The reaction for BNF is:

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\text{N}_2 + 8 \text{H}^+ + 8 \text{e}^- \rightarrow 2 \text{NH}_3 + \text{H}_2
\]

This type of reaction results in N\textsubscript{2} gaining electrons (see above equation) and is thus termed a reduction reaction. The exact mechanism of catalysis is unknown due to the technical difficulties biochemists have in actually visualizing this reaction in vitro, so the exact sequence of the steps of this reaction are not completely understood. Despite this, a great deal is known of the process. While the equilibrium formation of ammonia from molecular hydrogen and nitrogen has an overall negative enthalpy of reaction (i.e. it gives off energy), the energy barrier to activation is very high without the assistance of catalysis, which is done by nitrogenases. The enzymatic reduction of N\textsubscript{2} to ammonia therefore requires an input of chemical energy, released from ATP hydrolysis, to overcome the activation energy barrier.
A General Catalytic Mechanism Scheme for Nitrogenase: A) Components I and II are dissociated; II is ready for reduction. B) ATP binds to component II, which receives electrons from an electron donor (ferredoxin or flavodoxin); binding of ATP induces an allosteric conformational change which allows association of the two proteins. Electrons flow from the [4Fe-4S] cluster on II to the P cluster on I. C) Electrons are further shuttled to the iron-molybdenum cofactor (FeMoco), and ATP is hydrolysed to ADP. This step is repeated several times before a molecule of N₂ can bind to FeMoco. D) The protein complex dissociates, and nitrogenase reduces dinitrogen to ammonia and dihydrogen.

Legend: I: component I (dinitrogenase; MoFe protein); II: component II (dinitrogen reductase; Fe protein); ATP: adenosine triphosphate; ADP: adenosine diphosphate; Fdx: ferredoxin; Fld: flavodoxin.

Nitrogenase is made up of two soluble proteins: component I and II. Component I known as MoFe protein or nitrogenase contains 2 Mo atoms, 28 to 34 Fe atoms, and 26 to 28 acid-labile sulfides, also known as a iron-molybdenum cofactor (FeMoco). Component I is composed of two copies each of two subunits (α and β); each subunit’s stability depends on the other in vivo. Component II known as Fe protein or nitrogenase reductase is composed of two copies of a single subunit. This protein has four non-heme Fe atoms and four acid-labile sulfides (4Fe-4S). Substrate binding and reduction takes place on component I, which binds to ATP and ferredoxin or flavodoxin proteins (Fdx or Fld) (see step B).

The hydrolysis of ATP supplies the energy for the reaction while the Fdx/Fld proteins supply the electrons. Note this is a reduction reaction which means that electrons must be added to the N₂ to reduce it to NH₄. Thus, the role of component II is to supply electrons, one at a time to component I. ATP is not hydrolyzed to ADP until component II transfers an electron to component I (see step C and D). 21-25 ATPs are required for each N₂ fixed. The association of nitrogenase component I and II and later dissociation occurs several times to allow the fixation of one N₂ molecule (see step B and D).

Nitrogenase ultimately bonds each atom of nitrogen to three hydrogen atoms to form ammonia (NH₃). The nitrogenase reaction additionally produces molecular hydrogen as a side product, which is of special interest for people trying to produce H₂ as an alternative energy source to fossil fuels.

Key Points

- Nitrogen fixation does result in the release of energy, but the activation of this reaction takes energy in the form of ATP hydrolysis.
- Nitrogenases are metalloenzymes, which are proteins that have metallic molecules as subunits.
• While a great deal is known about how nitrogenases reduce nitrogen, some steps are unknown.

**Key Terms**

- **sulfide**: Any compound of sulfur and a metal or other electropositive element or group.
- **reduction**: A reaction in which electrons are gained and valence is reduced; often by the removal of oxygen or the addition of hydrogen.
- **enthalpy**: In thermodynamics, a measure of the heat content of a chemical or physical system.