Some Insights into How Nitrogenases Work

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Nitrogenase, the biological catalyst for N_2 fixation, occurs in widely diverse Eubacteria and Archaea, but not in Eukaryotes. It exists in three genetically distinct forms of the conventional nitrogenase, the well-known Mo-based system (Mo-nitrogenase) and two "alternative" systems, V-nitrogenase and Fe-nitrogenase. A fourth, completely unrelated Mo-based system also exists. All three conventional forms are not found in all N_2-fixing microorganisms. They all consist of two component proteins. The smaller component (the Fe protein or component-2), which is encoded by *nifH*, *vnfH* or *anfH*, respectively, is quite similar in all forms and occurs as a homodimer of ca. 63 kDa with a single [4Fe-4S] cluster bridging the subunits. It acts as an ATP-binding, specific electron donor to the larger protein component (the MoFe, VFe, FeFe protein, respectively, or component-1 of ca. 230 kDa), which is encoded by *nifDK*, *vnfDGK* or *anfDGK*, respectively, and contains the substrate-binding site. In heterologous crosses, not all components-2 can complement all components-1. For the Mo- and V-based systems (and likely for the Fe-nitrogenase), component 1 contains two copies of each of two types of prosthetic group; the P clusters and the FeMo(FeV)-cofactors (or M centers). Current dogma is that the P clusters operate as electron-acceptor/delivery systems for the bound cofactors at which N_2 fixation occurs.

After an initial comparison of the three nitrogenases and an outline of the catalyzed process, our recent efforts to gain both structural and mechanistic insights, based mainly on kineetic data derived from variant nitrogenases, a comparison of Mo- and V-nitrogenases and a new spectroscopic probe, will be presented. The effects of amino-acid substitutions in the polypeptide environment around the FeMo-cofactor on the catalytic and spectroscopic properties of the resulting variant nitrogenases will be analyzed as a probe of potential substrate (N_2 and C_2H_2) binding and reduction sites.

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