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Electrocatalytic CO_2 reduction catalyzed by nitrogenase MoFe and FeFe proteins

Bo Hu [a], Derek F. Harris [a], Dennis R. Dean [b], T. Leo Liu [a], Zhi-Yong Yang [a,*], Lance C. Seefeldt [a,*]

[a] Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322, USA
[b] Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061, USA

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ABSTRACT

Nitrogenases catalyze biological dinitrogen (N_2) reduction to ammonia (NH_3), and also reduce a number of non-physiological substrates, including carbon dioxide (CO_2) to formate ($HCOO^-$) and methane (CH_4). Three versions of nitrogenase are known (Mo-, V-, and Fe-nitrogenase), each showing different reactivities towards various substrates. Normally, electrons for substrate reduction are delivered by the Fe protein component of nitrogenase, with energy coming from the hydrolysis of 2 ATP to 2 ADP + 2 Pi for each electron transferred. Recently, it has been demonstrated that energy and electrons can be delivered from an electrode to the catalytic nitrogenase MoFe-protein without the need for Fe protein or ATP hydrolysis. Here, it is demonstrated that both the MoFe- and FeFe-protein can be immobilized as a polymer layer on an electrode and that electron transfer mediated by cobaltocene can drive CO_2 reduction to formate in this system. It was also found that the FeFe-protein diverts a greater percentage of electrons to CO_2 reduction versus proton reduction compared to the MoFe-protein. Quantification of electron flow to products exhibited Faradaic efficiencies of CO_2 conversion to formate of 9% for MoFe protein and 32% for FeFe-protein, with the remaining electrons going to proton reduction to make H_2.

1. Introduction

Nitrogenases catalyze all biological N_2 reduction and are the major contributor of fixed N into the biogeochemical N cycle [1]. There are three known types of nitrogenase, designated the Mo-dependent-, the V-dependent-, and the Fe-dependent-nitrogenase [2–6]. Each nitrogenase is coded for by distinct genes, resulting in distinct catalytic systems [7,8]. Nevertheless, the basic architecture for all the nitrogenases is similar; each requiring a distinct, but structurally similar, electron-donating Fe protein that supplies the electrons necessary for N_2 reduction in a series of one-electron transfers. In all of these systems, Fe protein delivers electrons to its corresponding catalytic partner, respectively designated the MoFe-, VFe-, or FeFe-protein, each of which house an electron mediator [8Fe-7S] cluster called P cluster and the active site metal cluster called FeMo-co, FeV-co, and FeFe-co, respectively [2,4,5,9] (Fig. 1). The active site structures for FeMo-co and FeV-co are known and are very similar, with exchange of Mo for V [10–15]. Further, the FeV-co contains a carbonate that replaces one of the bridging sulfides [10]. No X-ray crystal structure for the FeFe-co is available, but spectroscopic evidence indicates that it is similar to the other two cofactors with Fe substituting for V or Mo found in the other systems [16].

The catalytic cycle of nitrogenase involves the transient association of the Fe protein component with the catalytic component protein followed by the transfer of an electron from the Fe protein into the catalytic component with hydrolysis of two ATP molecules bound in the Fe protein to two ADP and two Pi [2,9,17,18]. The oxidized and ADP containing Fe protein dissociates and the cycle must be repeated for each electron accumulated [18–20]. Thus, the energy for electron accumulation at the active site for substrate reduction comes from hydrolysis of two ATP (ΔG° 61 kJ/mol) [9,21].

The reduction of substrates has been investigated for the Mo-dependent nitrogenase in considerable detail [2,9,17,18,22,23], while very little is known about the mechanism of the V- and Fe-nitrogenases [3–5,24]. For the Mo-nitrogenase, N_2 reduction requires 8 electrons and 8 protons, and thus the hydrolysis of 16 ATP (ΔG° 488 kJ/mol). One H_2 is formed for each N_2 reduced as an essential part of the catalytic mechanism [9,17,25–29]. In addition to reducing protons and N_2, Mo-nitrogenase has been shown to reduce a number of small molecules with double and triple bonds [2,30]. Among the many molecules reduced by Mo-nitrogenase is carbon dioxide (CO_2) [31–36]. There is considerable interest in reducing CO_2 as one way to mitigate CO_2 production from the burning of fossil fuels [37–41]. Mo-nitrogenase was shown to reduce CO_2 by two electrons and two protons

* Corresponding authors at: 0300 Old Main Hill, Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322, USA.
E-mail addresses: zhiyong.yang@aggiemail.usu.edu (Z.-Y. Yang), lance.seefeldt@usu.edu (L.C. Seefeldt).

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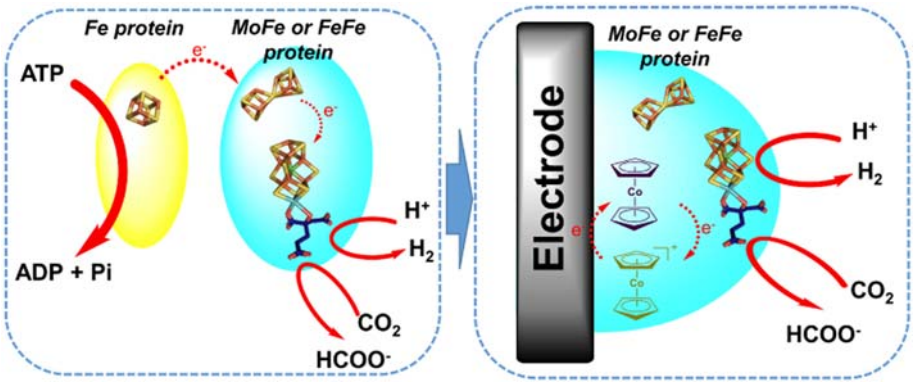

Fig. 1. Schematic showing nitrogenase catalysis with electron transfer from the Fe protein and ATP hydrolysis (left) or from cobaltocene mediated electron catalysis (right) for the MoFe or FeFe proteins catalyzing proton and CO_2 reduction.

to CO [31] and formic acid [33] and by 8 electrons and 8 protons to methane (CH_4) [32,34] (Eqs. (1)–(3)).

$$CO_2 + 2e^- + 2H^+ \rightarrow CO + H_2O \quad (1)$$

$$CO_2 + 2e^- + 2H^+ \rightarrow HCOOH \quad (2)$$

$$CO_2 + 8e^- + 8H^+ \rightarrow CH_4 + 2H_2O \quad (3)$$

The V-nitrogenase has also been shown to reduce CO_2 to CH_4 and C_2 and C_3 hydrocarbons [35,36]. Very recently, both in vivo and in vitro studies with the Fe-nitrogenase indicate that this enzyme shows the highest CO_2 reduction to CH_4 of the three nitrogenases [42]. This study demonstrated the reduction of CO_2 to CH_4 when driven by Fe protein and ATP for both purified Fe-nitrogenase component proteins and in whole cells.

As a way to bypass the energy requirement of ATP hydrolysis necessary for electron delivery by the Fe protein, an electrochemical method has been developed to allow the MoFe-protein to be immobilized in a polymer layer on a glassy carbon electrode with cobaltocene mediated electrocatalysis [43]. This method is a promising new approach to achieve small molecule reduction catalyzed by a nitrogenase component [43] and as a way to probe the mechanism of nitrogenase catalysis [44]. Using the MoFe-protein, it has been demonstrated that protons, nitrite, and azide can be reduced by electrons and energy supplied by an electrode [43,44]. Here, immobilization of the MoFe- and FeFe-proteins on a pyrene-stabilized bioelectrode was achieved to show catalytic reduction of CO_2 to formate. The FeFe-protein exhibits a higher Faradaic efficiency for CO_2 reduction when compared to the MoFe protein. The electron partition ratios between H_2 and formate production catalyzed by MoFe and FeFe protein are about the same as those obtained in the Fe protein supported reaction using ATP hydrolysis energy. These results provide evidence that the FeMo-co and FeFe-co in the corresponding nitrogenase proteins are the catalytic sites during electrocatalysis.

2. Materials and methods

2.1. Chemicals and general methods

Poly(vinylamine) hydrochloride (PVA) was purchased from Polysciences, Inc. (Washington, PA). Ethylene glycol diglycidyl ether (EDGDE) was received from TCI America (Portland, OR). Pyrene, sodium carbonate, bis(cyclopentadienyl)cobalt(III) hexafluorophosphate (cobaltocenium hexafluorophosphate) and 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were obtained from Sigma Aldrich (St. Louis, MO). All chemicals were used as received without further purification. Argon gas was purchased from Air Liquide America Specialty Gases LLC (Plumsteadville, PA) and was passed through an activated copper-catalyst to remove dioxygen contamination prior to use. Proteins and buffers were handled anaerobically in septum-sealed serum vials under an inert atmosphere (argon or dinitrogen), on a Schlenk vacuum line, or in anaerobic glove box (Teledyne Analytical Instruments, MO-10-M, Hudson, NH). The transfer of gases and liquids were done with gastight syringes. Glassy carbon electrodes were polished with 0.5 μm aluminum powder, sonicated in isopropanol and rinsed with deionized water before using. All electrochemical studies were done at room temperature in an Ar-filled glovebox ($[O_2] \leq 1$ ppm) using an Autolab PGSTAT 128 N potentiostat.

2.2. Bacterial growth and protein purification

Fe-nitrogenase proteins were expressed in *Azotobacter vinelandii* strain DJ1255 cells grown at 30 °C in Burk N-free medium with Na_2MoO_4 omitted in a custom-built 100 L fermenter with stirring and aeration to an OD_{600} of 1.8–2.0 and then harvested. Mo-nitrogenase proteins were expressed in *Azotobacter vinelandii* strains DJ995 for MoFe protein and DJ884 for Fe protein and purified as previously described [45]. Crude extracts were prepared and proteins purified according to previously described methods with minor modifications. Cell extracts of DJ1255 cells were prepared by using a French pressure cell operated at 1500 lb./in^2 in a degassed 50 mM Tris·HCl buffer (pH 8.0) with 2 mM sodium dithionite under Ar [46]. Protein concentrations were determined by the Biuret assay using bovine serum albumin as a standard. Protein purity was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis using Coomassie blue staining. The proteins were fully active based on proton reduction specific activities under Ar.

2.3. Fe protein/ATP-driven CO_2 reduction by MoFe and FeFe protein

CO_2 reduction assays were conducted in 9.4 mL serum vials containing an assay buffer consisting of an MgATP regeneration system (13.4 mM $MgCl_2$, 60 mM phosphocreatine, 10 mM ATP, 0.4 mg/mL creatine phosphokinase, and 1.2 mg/mL BSA) and 20 mM sodium dithionite in 100 mM HEPES buffer at pH 7.8. After solutions were made anaerobic, 0.45 atm CO_2 was added and the gas and liquid phases were allowed to equilibrate for approximately 10 min. FeFe or MoFe protein was then added, the vials vented to atmospheric pressure, and the reaction initiated by the addition of the appropriate Fe protein. Reactions were conducted at 30 °C for 60 min and then quenched by the addition of 500 μL of 400 mM EDTA pH 8.0. H_2 was quantified according to published methods [45]. Formate was quantified by two methods: (1) a previously described colorimetric assay [47] with an internal formate standard was prepared by adding $NaHCOO^-$ to 500 μL of sample to a final concentration of 2 mM; (2) a modified ^{1}H NMR method with