Redox Reactions of the Nitrogenase Complex from *Azotobacter vinelandii*†

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ABSTRACT: Purified nitrogenase complex from *Azotobacter vinelandii* behaves as a single protein entity during Sephadex G-200 chromatography, even though it consists of three different proteins (Fe protein, MoFe protein, and Shethna II protein) in a 1:1:1 ratio. This single entity behavior persists when the complex undergoes oxidation reactions with oxidants of different midpoint potentials. Up to 16 electrons can be removed from the complex from two different types of redox centers with no loss of enzymatic activity or change in metal content. Attempts to transfer all or part of these 16 easily removed electrons to reducible substrate to form reduced product and oxidized enzyme have been unsuccessful. The activity of the nitrogenase complex has been studied as a function of applied potential, and half-maximal activity was found at −465 mV.

Nitrogenase is the enzyme that catalyzes the six-electron reduction of dinitrogen to ammonia under ambient conditions. This enzyme system consists of two separately isolable proteins, the MoFe protein (M_r 230 000, 2 Mo atoms and 30 Fe atoms) and the Fe protein (M_r 65 000, 4 Fe atoms). Bulen & LeComte (1972) published methods for isolating a purified and apparently associated nitrogenase complex from *Azotobacter vinelandii* consisting of one MoFe protein, one Fe protein, and at least one and perhaps two Shethna II iron–sulfur proteins (Shethna et al., 1968). Haaker & Veeger (1977) and Scherings et al. (1977) have recently studied this complex from *A. vinelandii* and have reported that (1) it is associated in the presence of $S_2O_4^{2-}$ but dissociates in the presence of Mg^{2+} and (2) the Shethna protein is the component which imparts O_2 stability to this form of nitrogenase. Robson (1979) has studied the nitrogenase complex from *A. chroococcum* and found a 1:1:1 stoichiometry between the nitrogenase component proteins and an iron–sulfur protein which he showed also imparted O_2 stability to this form of nitrogenase. In the absence of Shethna protein or the equivalent protein in *A. chroococcum* the components are O_2 sensitive. Eady (1973) had earlier observed by ultracentrifuge measurements that the combined purified component proteins from *Klebsiella* are dissociated in the presence of 5 mM $Na_2S_2O_4$ but form a tight 1–1 complex in its absence. Extending these ultracentrifuge studies to the *A. chroococcum* proteins, Thorneley et al. (1975) reported identical results with those of Eady (1973).

The observations that, under certain conditions, the complex or component proteins can either associate or dissociate, plus the activity studies as a function of dilution for the complex (Silverstein & Bulen, 1970) or components (Thorneley et al., 1975), which indicate that below a certain protein concentration activity ceases, suggest an association–dissociation action for nitrogenase. Indeed, Hageman & Burris (1980a,b) have studied this aspect of nitrogenase catalysis extensively and propose a dynamic association–dissociation between the two component proteins during turnover. However, details of what occurs during the association interval are still unclear and remain problematical in developing a mechanism of nitrogenase catalysis. We have studied the redox properties of the purified nitrogenase complex and report here information which indicates that a significant interaction occurs between the component proteins comprising the nitrogenase complex from *A. vinelandii* when in the dye-oxidized state and during reduction of this dye-oxidized form. This protein interaction in the complex is sufficiently strong that the redox properties of the component proteins in the complex are altered relative to those in the free state.

Experimental Procedures

Methods. The nitrogenase complex in 0.05 M Tes,[1] 0.1 M NaCl, and 0.1 mg of $Na_2S_2O_4$ per mL from *A. vinelandii* OP was obtained by the method of Bulen & LeComte (1972). The specific activity of the complex was 250–320 nmol of H_2 min^{-1} (mg of total protein)$^{-1}$, and the Fe and Mo content ranged from 100 to 120 nmol/mg and from 5.7 to 6.2 nmol/mg, respectively. The visible spectrum of the nitrogenase complex in the presence of excess $Na_2S_2O_4$ was free of any cytochrome bands in the 550- and 420-nm regions.

Oxidized complex was obtained by reacting the nitrogenase complex (containing excess $Na_2S_2O_4$) with an excess of indigodisulfonate (IDS), methylene blue (MB), or dichlorophenolindophenol (DCPIP), allowing the reaction mixture to stand for various time intervals (usually 15–60 min), and then passing the protein–oxidant mixture through an anaerobic P-2 gel column to remove excess oxidant, its reduced form, SO_3^{2-}, etc. The oxidized nitrogenase complex was assayed for activity, analyzed for Fe and Mo, and then reduced with $Na_2S_2O_4$ in a polarographic cell as previously described (Watt et al., 1975) to measure total reducing equivalents transferred by $Na_2S_2O_4$ to oxidized complex. Controlled potential reduction of the oxidized nitrogenase complex as a function of potential was carried out by procedures previously described (Watt, 1979). All potentials were measured with reference to the saturated calomel electrode (SCE) but reported in the text referenced to the normal hydrogen electrode (NHE).

Reduced nitrogenase complex free of $S_2O_4^{2-}$ was prepared by passing the protein complex containing excess $Na_2S_2O_4$ through an anaerobic P-2 gel column prepared as described above. The absence of $S_2O_4^{2-}$ and the presence of fully reduced complex were verified by coulometric and polarographic methods.

Experiments designed to measure product formation and the redox state of the nitrogenase complex following partial

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[1] Abbreviations: Tes, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino]ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

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turnover of the reduced complex were set up as follows. Reduced, but $S_2O_4^{2-}$-free, nitrogenase complex (2–4 mL, 8–15 mg/mL) was placed in capped 10-mL assay vials which had been filled anaerobically with argon or 10% acetylene in argon. These vials were then incubated for 15 min at 30 °C following which 250-μL gas samples were removed and examined for H_2 and ethylene, respectively. Protein samples (50–100 μL) were also removed and injected into a coulometric cell controlled at −580 mV vs. NHE or into a polarographic cell where any change in $S_2O_4^{2-}$ concentration within the cell was monitored. Any gaseous products that formed or any change in the redox status of the protein during this incubation interval was determined. The anaerobic MgATP-generating system was then added (0.5–1.0 mL), and the mixture was incubated for 15 min following which the gas phase and the protein solution were examined as described above. Finally, limiting $S_2O_4^{2-}$ was added, the solution was incubated until all $S_2O_4^{2-}$ was consumed, and again the gas phase and solution were examined as outlined above.

The threshold potential for nitrogenase activity and the variation of this activity with variation in potential were determined as follows. The standard MgATP-generating system containing 1×10^{-4} M methyl- or benzylviologen as mediator and 0.1 M NaCl as electrolyte was reduced at a fixed potential in a previously described coulometry cell (Watt, 1979). When the 2.0 mL of MgATP-generating system–mediator solution reached the set potential, the small but constant residual current passing through the cell was recorded. Nitrogenase complex (0.1–0.5 mg) was then added to complete the assay mixture, and the resulting current was remeasured. When the potential of the complete assay mixture was sufficient to sustain enzyme turnover, a current flow larger than that prior to enzyme addition occurred, otherwise no change in current flow was observed. The current flow, i in microamperes (microcoulombs per second), under turnover conditions is related to micromoles of H_2 produced per second by eq 1 in

$$\mu\text{mol of } H_2/\text{s} = \frac{i}{(2)(96\,500)} \quad (1)$$

which 96 500 μC/μmol of electrons is the Faraday number. The specific activity is obtained by dividing eq 1 by the milligrams of protein used.

Results

Stepwise NaCl fractionation of the purified complex on DEAE-cellulose yields Shethna II protein (M_r 24 000), MoFe protein (M_r 230 000), and Fe protein (M_r 65 000) in an approximate 1:1:1 molar ratio. The metal analysis and activity of the purified complex also support this ratio. Furthermore, exclusion chromatography of the complex suggests a molecular mass in excess of 300 000 daltons. These results are in agreement with those of Scherings et al. (1977) and Veeger et al. (1980) and provide the basis for using 320 000 as the gram-molecular weight of the nitrogenase complex from *A. vinelandii*.

The nitrogenase complex maintains its original specific activity after oxidation with an excess of IDS, MB, or DCPIP and subsequent passage through an anaerobic P-2 gel column. No significant variation was observed in the Fe or Mo content between the nitrogenase complex as isolated and that following the dye-oxidation and gel filtration steps. The reductions of several preparations of dye-oxidized (MB or DCPIP) nitrogenase complex by $S_2O_4^{2-}$ in the polarographic cell are listed in Table I. A value near 16 electrons/mol of oxidized complex is obtained from $S_2O_4^{2-}$ reduction resulting in a value of ~0.5 electron/mol of Fe.

Table I: Reduction by $S_2O_4^{2-}$ of Five Separately Prepared Nitrogenase Complex Samples[a]

nitrogenase complex	μmol of Fe/mL	μmol of e added/mL	e/Fe	e/mol of complex
1	0.616	0.357	0.58	13.7
2	0.638	0.313	0.49	17.2
3	0.472	0.303	0.64	13.3
4	0.762	0.389	0.51	16.3
5	0.693	0.353	0.51	15.8

[a] The reduction by $S_2O_4^{2-}$ of five separately prepared nitrogenase complex samples which were oxidized with either methylene blue or DCPIP and chromatographed on anaerobic P-2 columns. The third column contains the micromoles of electrons per milliliter of complex transferred to the oxidized complex in the polarographic cell as measured by the decrease in $S_2O_4^{2-}$ concentration upon protein addition. The polarographic cell contained 2.0 mL of ~5×10^{-5} M $S_2O_4^{2-}$ to which several 50–100-μL aliquots of oxidized complex (4–6 mg/mL) were added. The value in column three is the average of these multiple additions.

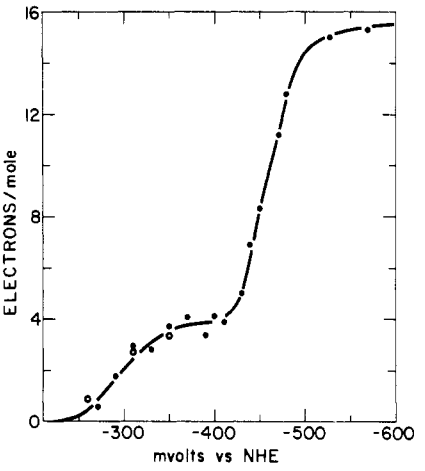

FIGURE 1: Coulometric reduction of oxidized nitrogenase complex as a function of potential. Methyl- and benzylviologens at 5×10^{-5} M in 0.05 M Tris and 0.1 M NaCl, pH 8.0, were the mediators used. (●) Reduction in the absence; (○) reduction in the presence of 1×10^{-3} M MgATP. Midpoint potentials and n values for the most positive and most negative redox centers are, respectively, −300 mV, 1.0 and −465 mV, 2.0.

Controlled potential electrolysis of this dye-oxidized nitrogenase complex as a function of potential is shown in Figure 1. Two redox regions are evident. The more positive one centered at −300 mV, which accepts 3–4 electrons/mol with an n value of 1, undergoes rapid methylviologen- or benzylviologen-mediated reduction at a platinum or mercury electrode. The more negative redox region, centered at −460 mV and accepting up to 12 electrons/mol with an n value of 2, undergoes a detectably slower reduction by the methyl- or benzylviologen mediator. The extent of reduction obtained from the limiting plateau at potentials more negative than −560 mV gave total reduction values near 16 electrons/mol in agreement with those obtained from $S_2O_4^{2-}$ reduction of the nitrogenase complex measured polarographically. Figure 1 also shows that there is no difference between reduction of the nitrogenase complex in the presence or absence of MgATP over the limited voltage region accessible to study. Potentials more negative than −350 mV could not be studied because the nitrogenase complex begins to turnover (see below), evolving H_2 from electrons mediated from the Pt electrode and hydrolyzing MgATP (Watt & Bulen, 1976; Evans & Albrecht, 1974; Hallenbeck, 1983; Braaksma et al., 1982). IDS oxidation of the complex produced an oxidized species which

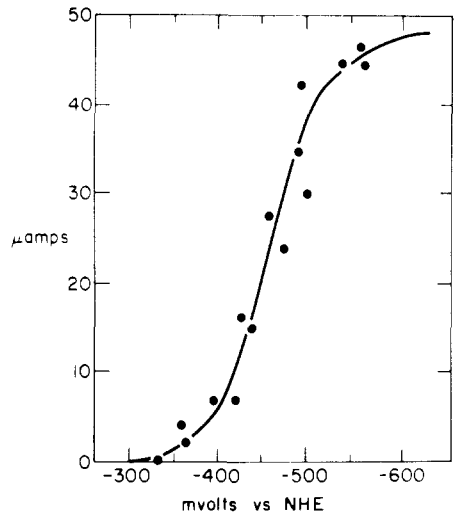

FIGURE 2: Current response in microamperes as a function of applied potential for the nitrogenase-catalyzed dihydrogen evolution reaction. The standard assay solution was used to which 1×10^{-4} M benzyl- or methylviologen was added as mediator. After equilibration at the selected potentials, 0.1 mg of nitrogenase complex was added to initiate the reaction. Least-squares analysis of the data points gives $E_{1/2} = -465$ mV and $n = 1.7$ for the nitrogenase-catalyzed reaction. Specific activities can be calculated from eq 1.

would only accept 3–4 electrons/mol as measured by $S_2O_4^{2-}$ uptake and only displayed the most positive redox center shown in Figure 1.

Figure 2 shows the activity of the nitrogenase complex in the presence of the MgATP-generating system as a function of applied potential. At potentials more positive than −300 mV, the nitrogenase complex does not accept electrons to produce H_2. However, at more negative potentials, a well-defined response of enzyme activity with decreasing potential is observed. The activity for the several different nitrogenase preparations studied follows a Nernstian redox curve with midpoint values near −460 mV and n values between 1.4 and 1.8. This latter value is difficult to measure with much precision because, as the potential becomes more negative, the nitrogenase-catalyzed reaction increases rapidly, causing the actual solution potential to lag slightly behind the set potential. The result of this effect is a somewhat lower activity than expected and, thus, a slight skewing of the curve in Figure 2 to the right.

The nitrogenase activity determined from eq 1 is usually 50–70% of that measured in the normal $S_2O_4^{2-}$-containing assay. Several factors, at present quantitatively unevaluated, may be contributing to this decrease. The first is that electrons are mediated from the Pt electrode to the protein by methyl- or benzylviologen. We have not optimized the mediator concentration for maximal activity nor have we demonstrated that these viologens are as effective in a normal assay as is $S_2O_4^{2-}$. The use of 0.1 M NaCl in the assay solution to lower solution resistance during electrolysis also causes a lowering of enzyme activity. Another factor, difficult to assess, is whether enzyme is adsorbed on the large area electrode during turnover, thus reducing its effective active site concentration and causing an apparently lower activity than expected. These factors and perhaps others need to be fully explored before a highly efficient electrochemical assay for nitrogenase is operational.

The first step of the sequential experiments involving the reaction of the reduced nitrogenase complex under atmospheres of argon and 10% acetylene in argon produced no measurable H_2 or ethylene, respectively. Neither redox state shown in Figure 1 was detected by controlled potential electrolysis or polarographic analysis following incubation under these gaseous atmospheres. The addition of anaerobic MgATP to reduced nitrogenase complex under argon or 10% acetylene in argon also produced no measurable hydrogen or ethylene nor did any oxidation of the nitrogenase complex occur as evidenced by polarographic or controlled potential electrolysis of the sample following incubation. However, upon addition of a known amount of $S_2O_4^{2-}$, hydrogen and ethylene were formed under argon and 10% acetylene in argon in amounts approaching those expected from the $S_2O_4^{2-}$ added (i.e., 1:1). Polarographic and controlled potential reduction of the enzyme sample following $S_2O_4^{2-}$ consumption indicated the enzyme was not in an oxidized state.

Discussion

The nitrogenase complex as isolated by the Bulen & LeComte (1972) method at low $S_2O_4^{2-}$ concentrations (~0.2 mM) and at low NaCl concentrations (0–0.1 M) behaves as a single entity even though three separate proteins of quite different size are present. Gel chromatography on Sephadex G-200 using low ionic strength elution buffers produces a nearly symmetrical, fully active protein fraction containing all three proteins. Our results are consistent with those of Veeger et al. (1980), who reported that chromatography of impure nitrogenase complex on Sepharose 4B produces an active protein band corresponding to 300 000 daltons. The observation that this three-component protein system moves easily on gel columns at ionic strength conditions where the MoFe protein normally crystallizes suggests that a rather strong complex is formed stabilizing the MoFe component from crystallization. The report by Scherings et al. (1977) indicating that chromatographic behavior on DEAE of the Shethna protein in the complex is significantly different from that of the free protein also supports a strong interaction of this protein in the complex. These authors report an approximate 1:1 stoichiometry for the Shethna protein and the nitrogenase proteins in their complex.

The metal analysis and the specific activity of the purified nitrogenase complex provide another basis for estimating the stoichiometry of the three proteins in the complex. The Fe and Mo analyses of several preparations give a range of 100–120 nmol of Fe and 5.6–6.2 nmol of Mo per mg of protein. The calculated values of 112 nmol of Fe/mg and 6.25 nmol of Mo/mg for these elements, assuming one Shethna protein (2 Fe), one Fe protein (4 Fe), and one MoFe protein (30 Fe, 2 Mo) for a total of 320 000 daltons, are in agreement with observed values and support a 1:1:1 stoichiometry. However, some ambiguity is still present particularly with regard to using the Fe content for this purpose because it is somewhat insensitive to the number of Shethna and Fe proteins present due to their low Fe content relative to that of the MoFe protein. For example, three Shethna and one Fe protein or one Shethna and two Fe proteins are also appropriate formulations with regard to Fe content, but only the former (besides the 1:1:1 ratio) is consistent with the Mo analysis.

The activity of 250–300 nmol of H_2 min^{-1} mg^{-1} for the nitrogenase complex can also be used for estimating the stoichiometry between the Fe and MoFe protein in the complex. Wherland et al. (1981) in their supplementary Table I give specific activity data for various combinations of purified *A. vinelandii* MoFe and Fe proteins. Their specific activities for 1:1 and 1.9:1 ratios are 381 ± 51 and 623 ± 33 nmol of H_2 min^{-1} mg^{-1} while that for the nitrogenase complex used in this study is 270–350 nmol of H_2 min^{-1} mg^{-1} (corrected for the presence of one Shethna protein per complex). A com-

parison of these two values with the specific activity of the complex used in this study suggests that the 1:1 stoichiometry is the more reasonable formulation.

The results just discussed for the *A. vinelandii* nitrogenase complex and those reported by Robson (1979) for the closely related *A. chroococcum* nitrogenase complex suggest that, at low ionic strengths and low $S_2O_4^{2-}$ concentrations, the nitrogenase preparation produced by the method of Bulen & LeComte (1972) exists as a tightly bound three-component protein complex with a most likely composition of one MoFe protein, one Fe protein, and one Shethna protein. The results discussed below are all based on this presumed 1:1:1 stoichiometry resulting in a calculated molecular weight of 320000 for the nitrogenase complex.

Table I and Figure 1 clearly show that the nitrogenase complex can be reversibly oxidized by as many as 16 electrons without loss of activity or change in metal content. Figure 1 further demonstrates that two different sets of redox centers undergo reduction after dye oxidation, one accounting for 3–4 electrons and the other for about 12 electrons. An easily discernible difference in the rate of reduction is also observed with reduction occurring more rapidly at the more positive centers and a slower reduction occurring with the more negative ones. We were unable to detect any change in the reduction behavior of the oxidized nitrogenase complex in the presence of the MgATP-generating system over the limited potential range corresponding to most of the first redox region of Figure 1. Enzyme turnover begins at potentials more negative than −300 mV and precludes further evaluation of any MgATP effect. The free Fe protein undergoes a redox reaction with an E of −290 mV in the absence of MgATP and one at −400 mV in its presence (Zumft et al., 1974). We have confirmed (unpublished results) these results with isolated Fe protein using the same electrochemical technique as was used for the nitrogenase complex in Figure 1. From arguments presented below, it seems reasonable that the oxidized Fe protein in the complex undergoes reduction at the −300 mV redox center in Figure 1, and consequently, we conclude that its redox potential is not shifted significantly by the presence of MgATP as is found in the free state. Perhaps the interaction of MgATP with free Fe protein, which shifts the E to a more negative value, is translated into other interactions when complexed with the other proteins of the nitrogenase complex. Further evaluation of this possibility from studies of a "synthetic complex" formed by recombining purified Fe protein, MoFe protein and Shethna II protein will be of interest.

A completely unequivocal assignment as to which of the three redox proteins known to comprise the nitrogenase complex is being reduced at a given potential in Figure 1 is not possible at this time. However, on the basis of known redox properties of the isolated component proteins, a reasonable proposal can be advanced.

The Fe protein and Shethna protein each contain one redox center and are present in a 1:1 or at most a 1:2 ratio in the nitrogenase complex. The Shethna protein undergoes only a one-electron oxidation while the Fe protein has been reported to be oxidized by both one (Ljones & Burris, 1972; Ljones, 1973) and two electrons (Thorneley et al., 1975). Both proteins have redox potentials near −300 mV, and both undergo rapid reduction in the coulometry cell. These properties are all consistent with the first redox center in Figure 1 (accepting a total of 3–4 electrons) corresponding to reduction of both the Shethna and Fe protein components of the nitrogenase complex. The lack of variation of the most positive centers in Figure 1 with MgATP is not consistent with known properties of the Fe protein but may be explained as discussed above.

The more negative E of Figure 1 involving up to 12 electrons is, therefore, likely to be the reduction of the MoFe protein component of the nitrogenase complex. Consistent with this view are reports (Watt & Bulen, 1976; Watt et al., 1980) that the isolated MoFe protein can be oxidized by as many as 12 electrons, undergoes reduction at a potential near that shown in Figure 1, and has a noticeably slow rate of reduction, similar to the rate of the slow-reduction component observed for reduction of the nitrogenase complex. Inconsistent with this view, however, is the report that, in the isolated 12-electron-oxidized MoFe protein, reduction occurs at two different potentials (Watt & Bulen, 1976; Watt et al., 1980), whereas Figure 1 shows reduction occurring at only one. The potential of the oxidized MoFe protein, thus, appears to behave differently in the nitrogenase complex than when isolated. This observation suggests that fairly strong interactions occur among the component proteins in the nitrogenase complex which affect the redox behavior of the MoFe protein. A systematic redox study of the purified MoFe protein in combination with both the purified Fe and Shethna proteins should produce details of this proposed interaction.

The large number of electrons that can be reversibly removed from the nitrogenase complex without altering its activity raises the question as to whether these electrons are available for substrate reduction. If so, it could be argued that by manipulating assay conditions, an oxidized state of the nitrogenase complex might be detected. Our efforts to induce the reduced complex to undergo a partial enzymatic turnover producing reduced substrate from these easily removed electrons contained in the complex and an oxidized form of the complex have been successful. Only when additional reductant is added to reduced complex along with MgATP does reduced substrate appear. Following consumption of the added reductant by the nitrogenase-catalyzed reaction, no oxidized form of the nitrogenase complex is observed. It appears then that substrate reduction by the complex occurs only when a more reduced state is attained, as is presently generally accepted, or that, at the very least, a "priming" of the enzyme occurs by addition of reducing equivalents. This event is then followed by a transfer of stored electrons from the complex to substrate to form oxidized enzyme and is concluded by a rapid rereduction to an enzyme form equivalent to the $S_2O_4^{2-}$-reduced state.

The potential required for half-nitrogenase activity has been previously reported for nitrogenase from *Chromatium*, −460 mV (Evans & Albrecht, 1974), from *A. vinelandii*, −460 mV (Watt & Bulen, 1976; Braaksma et al., 1982), and more recently with *Rhodopseudomonas capsulata*, −470 mV (Hallenbeck, 1983). The former and latter studies report n values of 2 for the Nernstian-type activity vs. potential curve, whereas our results give n values between 1.4 and 1.8. The n value is a parameter governing the steepness of the Nernstian curve in Figure 2. Very precise activity measurements and potential control are required to discriminate between n values of 1 and 2. A dynamic redox system which attempts to maintain a precisely fixed ratio of the oxidized and reduced forms of the enzyme reductant by balancing the rate of electrons "in" from an electrode (or chemical reductant) with those "out" to an actively turning-over redox enzyme is subject to numerous control problems especially at the extremes of the voltage regions of Figure 2. In addition, the uncertainties encountered with using a delicate enzyme such as nitrogenase

and maintaining a properly functioning MgATP-generating system in the presence of mediators make high precision measurements of the type required quite difficult. These problems would tend to lower the measured n value from that of the real value, and therefore, we conclude that the results in Figure 2 also support an n value of 2 for nitrogenase activity.

This n value is an important number because it is interpreted as the number of electrons that is transferred in an important redox reaction resulting in enzyme turnover. The question is, which of the two nitrogenase proteins is involved in this two-electron redox step? The Fe protein has been reported to undergo both a one- (Ljones & Burris, 1972; Ljones, 1973) and a two- (Thorneley et al., 1975; Braaksma et al., 1982) electron oxidation. Thus, it might be considered as a candidate for a two-electron redox reaction. However, the reported redox potentials of the isolated iron protein, both in the presence and in the absence of MgATP, and those shown in Figure 1 argue against the Fe protein being the $n = 2$ redox protein in Figure 2. The redox potential of −300 mV for the Fe protein in the absence of MgATP and −400 mV in the presence of MgATP are 160 and 60 mV too positive, respectively, to sustain nitrogenase reduction as observed in Figure 2. The reported turnover potential of −485 to −495 mV (Scherings et al., 1977) with *A. vinelandii* nitrogenase and flavoprotein further suggests that the operating potential for nitrogenase in this system is even further outside the range of known Fe protein redox properties. We, therefore, conclude that redox centers on the MoFe protein are responsible for the redox activity shown in Figure 2. Figure 1 indicates that there are redox centers in the nitrogenase complex, presumably on the MoFe protein, with midpoint potential coincident with that shown in Figure 2. Also, in isolated MoFe protein, redox centers identified as the so-called "p clusters" (Zimmerman et al., 1978) undergo reduction at a potential of −480 mV (Watt et al., 1980). Experiments utilizing the purified Fe and MoFe protein components may be helpful in further understanding this redox behavior.

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