The vanadium- and molybdenum-containing nitrogenases of *Azotobacter chroococcum*

Comparison of mid-point potentials and kinetics of reduction by sodium dithionite of the iron proteins with bound magnesium adenosine 5'-diphosphate

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The mid-point potentials of the Fe protein components (Ac2 and Ac2* respectively) of the Mo nitrogenase and V nitrogenase from *Azotobacter chroococcum* were determined in the presence of MgADP to be -450 mV (NHE) [$\text{Ac2}(\text{MgADP})_2$ - $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ couple] and -463 mV (NHE) [$\text{Ac2}^*(\text{MgADP})_2$ - $\text{Ac2}^*_{\text{ox}}(\text{ADP})_2$ couple] at 23°C at pH 7.2. These values are consistent with a flavodoxin characterized by Deistung & Thorneley [(1986) *Biochem. J.* **239**, 69–75] with $E_m = -522$ mV (NHE) being an effective electron donor to both the Mo nitrogenase and the V nitrogenase *in vivo*. $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ and $\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2$ were reduced by SO_2^{2-} (formed by the predissociation of dithionite ion, $\text{S}_2\text{O}_4^{2-}$) at similar rates, $k = 4.7 \times 10^6 \pm 0.5 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ and $3.2 \times 10^6 \pm 0.2 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ respectively, indicating structural homology at the electron-transfer site associated with the [4Fe-4S] centre in these proteins.

INTRODUCTION

The V nitrogenase system in *Azotobacter chroococcum* is genetically distinct from the Mo nitrogenase system that is normally expressed in this organism when grown on nitrogen-free medium containing molybdenum (Robson *et al.*, 1986a).

The construction of a strain of *A. chroococcum* (M.C.D. 1155) in which the structural genes encoding Mo nitrogenase (*nifHDK*) are deleted (i.e. the Fe and MoFe proteins cannot be synthesized) facilitated the isolation and characterization of the two proteins that comprise the V nitrogenase (Robson *et al.*, 1986a; Eady *et al.*, 1987). The tetrameric VFe protein (Ac1*), M_r 210000, contains 2 V atoms, 23 Fe atoms and 20 acid-labile sulphide groups per molecule (Eady *et al.*, 1987). The Fe protein (Ac2*) has M_r 60000 (Robson *et al.*, 1986a) and contains 4 Fe atoms and 4 acid-labile sulphide groups per molecule.

The Mo nitrogenase from *A. chroococcum* is similar to nitrogenases isolated from several diazotrophs and comprises an MoFe protein (Ac1), M_r 227000, with 1.9 ± 0.3 Mo atoms, 23 ± 2 Fe atoms and 20 ± 2 acid-labile sulphide groups per molecule, and an Fe protein (Ac2), M_r 64000, with 4 Fe atoms and 4 acid-labile sulphide groups per molecule (Yates & Planque, 1975). Since Ac2 is encoded by *nifH*, a gene that has only 89% sequence homology with the *nifH** gene encoding Ac2*, these Fe proteins are clearly distinct (Robson *et al.*, 1986b).

The V nitrogenase, like the Mo nitrogenases, re-

quires MgATP and a source of low-potential electrons ($\text{Na}_2\text{S}_2\text{O}_4$ *in vitro*) to reduce substrates such as H^+ , N_2 and C_2H_2 (Eady *et al.*, 1987). The limited amount of steady-state kinetic and spectroscopic data published for the purified V nitrogenases from *A. chroococcum* (Eady *et al.*, 1987; Dilworth *et al.*, 1988) and *Azotobacter vinelandii* (Hales *et al.*, 1986a,b) suggests the same sequence of electron-transfer reactions and a similar overall mechanism to that proposed by Lowe & Thorneley (1984a, and references cited therein) for Mo nitrogenase from *Klebsiella pneumoniae*. The differences in substrate specificity (i.e. V nitrogenase reduces C_2H_2 to $\text{C}_2\text{H}_4 + \text{C}_2\text{H}_6$ whereas Mo nitrogenase reduces C_2H_2 only to C_2H_4 ; Dilworth *et al.*, 1988) are due to the different reactivities of V and Mo at the substrate-reduction site. Our current understanding of the detailed kinetics and mechanism of nitrogenase action is based largely on computer simulations using independently determined rate constants for the partial reactions that comprise the catalytic cycle of the Mo nitrogenase from *K. pneumoniae* [see Thorneley & Lowe (1985) for review and Orme-Johnson (1985) for an appraisal of this approach]. We have therefore commenced a study of the analogous reactions with both the V nitrogenase and Mo nitrogenase component proteins from *A. chroococcum*. In the present paper we described the kinetics of the first reaction in the electron-transfer sequence, the reduction of oxidized Fe protein with MgADP bound by $\text{Na}_2\text{S}_2\text{O}_4$. This form of the Fe protein is generated as a product of the MgATP-dependent electron-transfer reaction from Fe protein to MoFe protein or VFe protein in the

Abbreviations used: we refer to the protein components as Ac1* for the VFe protein and Ac2* for the Fe protein from *Azotobacter chroococcum*, where the asterisk distinguishes these proteins from the nitrogenase from molybdenum-sufficient cultures of the parent strain; Kp1 and Kp2 and Av1 and Av2 are the MoFe protein and Fe protein from *Klebsiella pneumoniae* and *Azotobacter vinelandii* respectively; E_m is the mid-point potential relative to the normal hydrogen electrode (NHE).

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Fig. 1. Stopped-flow amplitude titrations at 420 nm for the reduction of $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ and $\text{Ac2}^*(\text{MgADP})_2$ by $\text{SO}_2^{\cdot-}$ at various potentials at 23 °C at pH 7.2

The potential was set by adding Na_2SO_3 (0–180 mM) to $\text{Na}_2\text{S}_2\text{O}_4$ (4 mM) contained in one syringe of the stopped-flow apparatus. The other syringe contained either Ac2_{ox} (44 μM) or Ac2^* (46 μM). Both syringes contained ADP (1 mM), MgCl_2 (10 mM) and Hepes buffer, pH 7.2 (25 mM). The inset shows the calibration curve used to determine the final potentials by replacing the proteins by methyl viologen (25 μM after mixing), $E_m = -446$ mV (NHE). ●, $\text{Ac2}^*(\text{MgADP})_2$, $E_m = -463$ mV (NHE); ■, $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$, $E_m = -450$ mV (NHE). The lines are least-squares best fits to the Nernst equation with $n = 1.0$.

Fe protein cycle described by Thorneley & Lowe (1983) for Kp nitrogenase. The mid-point potentials (E_m) for the couples $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ – $\text{Ac2}_{\text{red}}(\text{MgADP})_2$ and $\text{Ac2}^*(\text{MgADP})_2$ – $\text{Ac2}_{\text{red}}(\text{MgADP})_2$ were determined and are compared with the E_m for a flavodoxin that is considered to be the electron donor to nitrogenase in *A. chroococcum* (Yates, 1972; Deistung & Thorneley, 1986).

MATERIALS AND METHODS

Ac2 and Ac2^* were purified and assayed as previously described (Robson *et al.*, 1986a) to specific activities of 1380 and 1340 nmol of H_2 produced/min per mg of protein respectively. Both proteins contained 3.7 ± 0.2 g-atoms of Fe/mol of protein. Oxidized proteins were prepared by using Indigo Carmine dye bound to Dowex AG1-X8 resin as previously described (Ashby & Thorneley, 1987).

All reactions were studied at 23 °C in 25 mM-Hepes buffer, pH 7.2, containing 10 mM- MgCl_2 , 1 mM-ADP and various concentrations of $\text{Na}_2\text{S}_2\text{O}_4$, Na_2SO_3 and Na_2SO_4 . $\text{Na}_2\text{S}_2\text{O}_4$ was shown to be 80% pure by spectrophotometric titration with $\text{K}_3\text{Fe}(\text{CN})_6$. The 20% impurity was assumed to be Na_2SO_3 .

All biochemicals were purchased from Sigma Chemical Co., Poole, Dorset, U.K., and salts from BDH Chemicals, Poole, Dorset, U.K.

Stopped-flow spectrophotometry was performed with an Aminco apparatus installed in a high-integrity glove box through which N_2 gas was circulated and an O_2 concentration less than 1 p.p.m. maintained (Thorneley & Lowe, 1983).

The stopped-flow data were recorded and analysed on

a Digital Equipment Co. PDP1134A computer interfaced to the stopped-flow spectrophotometer via a BBC model B micro computer. Rate constants were obtained by fitting the recorded data to exponential functions by using least-squares minimization as previously described (Thorneley & Lowe, 1983).

Mid-point potentials were obtained by fitting the amplitudes of absorption changes at various potentials to the Nernst equation as previously described (Deistung & Thorneley, 1986).

RESULTS AND DISCUSSION

Mid-point potentials for $\text{Ac2}^*(\text{MgADP})_2$ and $\text{Ac2}(\text{MgADP})_2$

The determination of mid-point potentials for nitrogenase Fe protein is complicated because: (i) two interconvertible Fe-S cluster conformations exist for the reduced state, with $S = \frac{1}{2}$ and $S = \frac{3}{2}$ spin systems [see Lindahl *et al.* (1985), Hagen *et al.* (1985) and Watt & McDonald, (1985); for studies with Av2 and Kp2, similar signals have been observed for both Ac2 and Ac2^* (D. J. Lowe, unpublished work)]; (ii) E_m values obtained at low temperatures by the use of e.p.r. spectroscopy may differ from room-temperature values; (iii) the binding of MgATP or MgADP decreases the mid-point potentials by as much as 140 mV relative to that of the nucleotide-free proteins (Watt, 1985).

Stopped-flow spectroscopy at 420 nm using the change in absorbance of the [4Fe-4S] centre on oxidation–reduction and setting the potential with the $\text{SO}_2^{\cdot-}$ – SO_3^{2-} couple has some advantages over other equilibrium, spectroscopic and electrochemical methods. Firstly, it avoids the use of dye mediators and problems associated

Fig. 2. Kinetics of reduction of $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ and $\text{Ac2}^*(\text{MgADP})_2$ by $\text{Na}_2\text{S}_2\text{O}_4$ at 23 °C at pH 7.2

The data were obtained by stopped-flow spectrophotometry at 420 nm and with the kinetic analysis described in the Materials and methods section. Syringe A contained Ac2_{ox} (44 μM) or Ac2^* (46 μM). Syringe B contained $\text{Na}_2\text{S}_2\text{O}_4$ (1.6–72 mM). Both syringes contained ADP (1 mM), MgCl_2 (10 mM) and Hepes buffer, pH 7.2 (25 mM). k_{obs} is the observed first-order rate constant for the single-exponential change in absorbance at 420 nm that occurs when the oxidized proteins are reduced by $\text{S}_2\text{O}_4^{2-}$ in the presence of MgADP. $\blacktriangle$, Ac2_{ox} ; $\bullet$, Ac2^* . The inset shows a typical trace obtained with $[\text{S}_2\text{O}_4^{2-}] = 4$ mM (after mixing) for $\text{Ac2}^*(\text{MgADP})_2$. The noise-free line is the best-fit single exponential with $k_{\text{obs}} = 8.15 \text{ s}^{-1}$.

with protein adsorption on electrode surfaces. Secondly, it allows measurements to be made at room temperature rather than at the low temperatures associated with e.p.r. spectroscopy of Fe-S centres. Thirdly, it allows the kinetics of the approach to equilibrium to be monitored and offers the possibility of detecting transient species and/or heterogeneity in either reactants or products. This latter possibility is particularly important in the present case because of the existence of the two forms of [4Fe-4S] cluster in the reduced state ($S = \frac{1}{2}$ and $S = \frac{3}{2}$) and the conclusions of Lowe & Thorneley (1984) and Ashby & Thorneley (1987) that Fe proteins with specific activities of approx. 1500 nmol of H_2 produced/min per mg of protein are only 40% active with respect to electron transfer to MoFe protein.

Fig. 1 shows stopped-flow amplitude data for the reduction of $\text{Ac2}^*(\text{MgADP})_2$ (23 μM) at various final potentials set by addition of Na_2SO_3 (0–180 mM). The potential of each solution after reaction was determined in a parallel experiment in which methyl viologen dication (25 μM) substituted for the proteins with the monitoring wavelength changed from 420 to 602 nm ($\Delta\epsilon_{602} = 12 \text{ mm}^{-1} \cdot \text{cm}^{-1}$; Thorneley, 1974). An $E_m = -446$ mV for the methyl viologen dication–monocation couple (Mayhew, 1978) was used to construct the calibration curve shown as an inset to Fig. 1. The measured potentials of the solutions were approx. 5 mV more negative than those calculated by using the equations and constants given by Mayhew (1978). This difference is easily accounted for by the uncertainty in the extent of Na_2SO_3 contamination (assumed to be 20%) of the commercially available $\text{Na}_2\text{S}_2\text{O}_4$ [80% purity by $\text{K}_3\text{Fe}(\text{CN})_6$ titration].

The addition of Na_2SO_3 to set the potential involves an increase in ionic strength. The effect of this was

investigated at a potential close to the E_m for both proteins by addition of redox-inactive Na_2SO_4 (35 mM) to $\text{Na}_2\text{S}_2\text{O}_4$ (1 mM) plus Na_2SO_3 (20 mM) (final mixed concentrations). This did not change the amplitude and it was concluded that there is no detectable ionic-strength effect on the redox equilibrium. This is consistent with the excellent fit of the data points in Fig. 1 to the Nernst equation over a range of potentials when the ionic strength is changing.

The amplitudes used in the construction of Fig. 1 were associated with a single-exponential process (see inset to Fig. 2) that was completely observable within the time range accessible to the stopped-flow apparatus, i.e. no fast phase(s) occurring within the mixing time or slow phase(s) occurring over a period of minutes. This contrasts with earlier studies when multiple phases were observed with $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ of a similar specific activity that had been prepared by oxidation with phenazine methosulphate without anaerobic glove-box facilities (Yates *et al.*, 1975).

The E_m values calculated by using the Nernst equation with $n = 1$ are -450 and -463 mV for $\text{Ac2}(\text{MgADP})_2$ and $\text{Ac2}^*(\text{MgADP})_2$ respectively. Allowing the n value to float caused E_m to shift by at most 0.5 mV with a deviation of 0.05 in the value of n . We conclude that the reductions of $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ and $\text{Ac2}^*(\text{MgADP})_2$ are one-electron processes and that the environment and geometry of the [4Fe-4S] cluster, inasmuch as these determine mid-point potentials, are very similar in both proteins. Watt (1985) determined $E_m = -480$ mV for $\text{Av2}(\text{MgADP})_2$ by using microcoulometry. Thus the *Azotobacter* Fe proteins have significantly lower potentials than the corresponding forms from *K. pneumoniae* ($E_m = -398$ mV) (Deistung & Thorneley, 1986) and

Clostridium pasteurianum ($E_m = -380$ mV) (Zumft *et al.*, 1974). This is reflected in the E_m values for flavodoxins from the organisms that serve as the electron donors to nitrogenase *in vivo*.

A flavodoxin ($E_m = -422$ mV), the product of the *nifF* gene in *K. pneumoniae*, mediates electron transfer from the *nifJ*-gene product, a pyruvate flavodoxin oxidoreductase, to $\text{Kp2}_{\text{ox}}(\text{ADP})_2$ (Hill & Kavanagh, 1980; Shah *et al.*, 1983; Deistung & Thorneley, 1986). In *A. chroococcum* and *A. vinelandii* it is also likely that flavodoxins mediate electron transfer to nitrogenase, but the nature of the donor to flavodoxin is uncertain [see Deistung & Thorneley (1986) and references cited therein for a review of the recent literature]. Yates (1972) isolated a flavodoxin from *A. chroococcum* that Deistung & Thorneley (1986) showed had $E_m = -522$ mV for the semiquinone-hydroquinone couple at pH 7.5. The E_m values for $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ (-450 mV) and $\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2$ (-463 mV) would allow this flavodoxin to serve as an effective electron donor to both the Mo- and V-containing nitrogenases in *A. chroococcum*, i.e. both nitrogenases could utilize the same electron-transport system.

Kinetics of reduction of $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ and $\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2$ by $\text{Na}_2\text{S}_2\text{O}_4$ at 23 °C at pH 7.2

Ac2_{ox} and Ac2^*_{ox} were prepared as described above. When either protein + MgADP (1 mM) was mixed in the stopped-flow apparatus with $\text{Na}_2\text{S}_2\text{O}_4$ (1.6–72 mM) plus MgADP (1 mM) a single-exponential function described the decrease in absorbance at 420 nm. Fig. 2 shows the dependence of the observed first-order rate constant (k_{obs}) on $[\text{S}_2\text{O}_4^{2-}]^{\frac{1}{2}}$. Increasing the concentration of MgADP to 8 mM (after mixing) did not change k_{obs} , or the amplitude, indicating saturation binding of MgADP to the proteins at 1 mM-MgADP. The half-order dependence on $\text{S}_2\text{O}_4^{2-}$ concentration is due to the pre-dissociation reaction (eqn. 1) that yields the active reductant $\text{SO}_2^{\cdot-}$ (eqn. 2):

$$\frac{d[\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2]}{dt} = k_{+1}K^{\frac{1}{2}}[\text{S}_2\text{O}_4^{2-}]^{\frac{1}{2}}[\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2] \quad (3)$$

Under the conditions of the present experiments and neglecting the back reaction (k_{-1}), Lambeth & Palmer (1973) and Ashby & Thorneley (1987) showed that eqn. (3) applies with $k_{\text{obs}} = k_{+1}K^{\frac{1}{2}}[\text{S}_2\text{O}_4^{2-}]^{\frac{1}{2}}$. Thorneley & Lowe (1983) determined $K = 1.6 \times 10^{-9} \pm 0.2 \times 10^{-9}$ M under the conditions of the present experiments.

The linear dependence of k_{obs} on $[\text{S}_2\text{O}_4^{2-}]^{\frac{1}{2}}$ required by eqn. (3) was observed up to $[\text{S}_2\text{O}_4^{2-}] = 4$ mM with $k_{+1} = 3.2 \times 10^6 \pm 0.2 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ and $4.7 \times 10^6 \pm 0.5 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ for $\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2$ and $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ respectively. We attribute the deviation from linearity that occurs with both proteins at $[\text{S}_2\text{O}_4^{2-}] > 4$ mM (Fig. 2) to two opposing effects: (i) inhibition of the rate of reduction at high ionic strength, and (ii) incomplete reduction of the proteins because of the rise in redox potential that occurs with increasing $\text{S}_2\text{O}_4^{2-}$ concentration.

A pronounced ionic-strength effect on the value of

Fig. 3. Effect of ionic strength on the rate of reduction of $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ and $\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2$ at 23 °C at pH 7.2

The data were obtained by stopped-flow spectrophotometry. Syringe A contained either Ac2_{ox} (50 μM) or Ac2^*_{ox} (50 μM). Syringe B contained $\text{Na}_2\text{S}_2\text{O}_4$ (2 mM) and various concentrations of Na_2SO_4 (0–200 mM). Both syringes contained ADP (2 mM), MgCl_2 (10 mM) and Hepes buffer, pH 7.2 (25 mM). k_{obs} is the observed first-order rate constant for the single-exponential change in absorbance at 420 nm that occurs when the oxidized proteins are reduced by $\text{S}_2\text{O}_4^{2-}$ in the presence of MgADP. ▲, Ac2_{ox} ; ●, Ac2^*_{ox} .

k_{obs} for both $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ and $\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2$ was shown by the addition of redox-inactive Na_2SO_4 (Fig. 3). This effect is not due to a dependence of K (eqn. 1) on ionic strength, since Thorneley & Lowe (1983) showed by e.p.r. spectroscopy that the concentration of $\text{SO}_2^{\cdot-}$ in a 10 mM- $\text{Na}_2\text{S}_2\text{O}_4$ solution decreased by only 7% when 200 mM- Na_2SO_4 was added. Although Na_2SO_4 decreased k_{obs} , it did not change the amplitude, indicating complete reduction of both proteins by $\text{Na}_2\text{S}_2\text{O}_4$ (1.5 mM) at high ionic strength. However, increasing $[\text{Na}_2\text{S}_2\text{O}_4]$ from 0.8 to 40 mM in the absence of Na_2SO_4 caused the amplitudes to decrease by 36% for $\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2$ and by 28% for $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$. Mayhew (1978) showed that increasing $[\text{Na}_2\text{S}_2\text{O}_4]$ from 0.8 to 40 mM increases the potential of a solution by 49 mV, this being due to the effect of the pre-equilibrium shown in eqn. (1). This effect, together with Na_2SO_3 impurity (20% by weight) in the $\text{Na}_2\text{S}_2\text{O}_4$, caused a solution made up to be 50 mM- $\text{Na}_2\text{S}_2\text{O}_4$ (actually 40 mM- $\text{Na}_2\text{S}_2\text{O}_4$ /14 mM- Na_2SO_3) to have a potential of -480 mV (determined by the extent of reduction of 25 μM -methyl viologen monocation, monitored at 602 nm). The E_m values for $\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2$ (-463 mV) and for $\text{Ac2}_{\text{ox}}(\text{MgADP})_2$ (-450 mV) require these proteins to be 34% and 25% oxidized at a potential of -480 mV. This is entirely consistent with the observed decrease in amplitude on increasing $[\text{Na}_2\text{S}_2\text{O}_4]$ from 0.8 to 40 mM. The kinetics of the approach to equilibrium with $[\text{Na}_2\text{S}_2\text{O}_4] > [\text{Na}_2\text{SO}_3] \gg [\text{Ac2}^*_{\text{ox}}(\text{MgADP})_2]$ are described by a single-exponential process with $k_{\text{obs}} = k_{+1}[\text{SO}_2^{\cdot-}] + k_{-1}[\text{SO}_2]$ [see Espenson (1981) for a kinetic analysis of equilibrium processes]. Thus incomplete reduction of the proteins at high dithionite concentrations should cause k_{obs} to

increase relative to the value predicted by eqn. (3). The downward curvature in Fig. 2 means that the decrease in k_{+1} due to the effect of ionic strength more than compensates for the term $k_{-1}[\text{SO}_2]$ that is responsible for partial reduction of the proteins. These effects preclude any interpretation of the curvature in the data at high $\text{S}_2\text{O}_4^{2-}$ concentration in terms of a rate-limiting first-order process such as a protein conformation change or internal electron transfer following pre-equilibrium binding of SO_2 to the protein.

The values of $k_{+1} = 3.2 \times 10^6 \pm 0.2 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ and $4.7 \times 10^6 \pm 0.5 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ for $\text{Ac}2_{\text{ox}}^*(\text{MgADP})_2$ and $\text{Ac}2_{\text{ox}}(\text{MgADP})_2$ respectively are very close to that of $3.0 \times 10^6 \pm 0.4 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ reported for $\text{Kp}2_{\text{ox}}(\text{MgADP})_2$ by Thorneley & Lowe (1983). However, they found that increasing the ionic strength by the addition of Na_2SO_4 (50 mM) to $\text{Na}_2\text{S}_2\text{O}_4$ (1 mM) had no effect on the rate of reduction of $\text{Kp}2_{\text{ox}}(\text{MgADP})_2$. This may indicate a different charge distribution at the site of electron transfer on the Fe proteins of *K. pneumoniae* and *A. chroococcum*.

CONCLUSIONS

The mid-point potentials and kinetic data in the present paper provide further evidence for a similarity in the overall mechanism of action of the Mo nitrogenase and V nitrogenase systems in *A. chroococcum* and are consistent with a flavodoxin, $E_m = -522 \text{ mV}$, being a competent electron donor to both systems *in vivo*.

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REFERENCES

- Ashby, G. A. & Thorneley, R. N. F. (1987) *Biochem. J.* **246**, 455–465
 Deistung, J. & Thorneley, R. N. F. (1986) *Biochem. J.* **239**, 69–75
 Dilworth, M. J., Eady, R. R. & Eldridge, M. E. (1988) *Biochem. J.* **249**, 745–751
 Eady, R. R., Robson, R. L., Richardson, T. H., Miller, R. W. & Hawkins, M. (1987) *Biochem. J.* **244**, 197–207
 Espenson, J. H. (1981) in *Chemical Kinetics and Reaction Mechanism*, pp. 42–45, McGraw-Hill, New York
 Hagen, W. R., Dunham, W. R., Braaksma, A. & Haaker, H. (1985) *FEBS Lett.* **187**, 146–150
 Hales, J. B., Case, E. E., Morningstar, J. E., Dzeda, M. F. & Mauterer, L. A. (1986a) *Biochemistry* **25**, 7251–7255
 Hales, J. B., Langosch, D. J. & Case, E. E. (1986b) *J. Biol. Chem.* **261**, 15301–15306
 Hill, S. & Kavanagh, E. P. (1980) *J. Bacteriol.* **141**, 470–475
 Lambeth, D. O. & Palmer, G. (1973) *J. Biol. Chem.* **248**, 6095–6103
 Lindahl, P. A., Day, E. P., Kent, T. A., Orme-Johnson, W. H. & Münck, E. (1985) *J. Biol. Chem.* **260**, 11160–11173
 Lowe, D. J. & Thorneley, R. N. F. (1984) *Biochem. J.* **224**, 877–886
 Mayhew, S. G. (1978) *Eur. J. Biochem.* **85**, 535–547
 Orme-Johnson, W. H. (1985) *Annu. Rev. Biophys. Chem.* **14**, 419–459
 Robson, R. L., Eady, R. R., Richardson, T. H., Miller, R. W., Hawkins, M. & Postgate, J. R. (1986a) *Nature (London)* **322**, 388–390
 Robson, R. L., Woodley, P. & Jones, R. (1986b) *EMBO J.* **5**, 1159–1163
 Shah, V. K., Stacey, G. & Brill, W. J. (1983) *J. Biol. Chem.* **258**, 12064–12068
 Thorneley, R. N. F. (1974) *Biochim. Biophys. Acta* **333**, 487–496
 Thorneley, R. N. F. & Lowe, D. J. (1983) *Biochem. J.* **215**, 393–403
 Thorneley, R. N. F. & Lowe, D. J. (1985) *Met. Ions Biol.* **7**, 221–284
 Watt, G. D. (1985) in *Nitrogen Fixation Research Progress* (Evans, H. J., Bottomley, P. J. & Newton, W. E., eds.), pp. 585–590, Martinus Nijhoff, Dordrecht and Boston
 Watt, G. D. & McDonald, J. W. (1985) *Biochemistry* **24**, 7226–7231
 Yates, M. G. (1972) *FEBS Lett.* **27**, 63–67
 Yates, M. G. & Planque, K. (1975) *Eur. J. Biochem.* **60**, 467–476
 Yates, M. G., Thorneley, R. N. F. & Lowe, D. J. (1975) *FEBS Lett.* **60**, 89–93
 Zumft, W. G., Mortenson, L. E. & Palmer, G. (1974) *Eur. J. Biochem.* **46**, 525–535

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