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Introduction

Hydrogenases are enzymes which catalyze reactions involving dihydrogen. They serve integral roles in a number of microbial metabolic pathways. As one example, hydrogenases oxidize the H_2 generated by nitrogenase in biological N_2 fixation. This "recycling" of H_2 leads to an increased efficiency of N_2 fixation. Our research is focussed on investigations of the catalytic mechanism of the hydrogenases found in aerobic, N_2-fixing microorganisms such as *Azotobacter vinelandii* and the agronomically important *Bradyrhizobium japonicum* as well as microorganisms with similar hydrogenases. The hydrogenases isolated from these microorganisms are Ni- and Fe-containing heterodimers. Our work has focussed on three areas during the last grant period. In all cases, a central theme has been the role of inhibitors in the characteristics under investigation. In addition, a number of collaborative efforts have yielded interesting results.

Investigations of Inhibitor Mechanisms

In metalloenzymes such as hydrogenase, inhibitors often influence the activity of the enzyme through ligand interactions with redox centers, often metals, within the enzyme. Therefore, investigations of the ability of various compounds to inhibit an enzyme's activity, as well as the mechanism of inhibition, can provide insight into the catalytic mechanism of the enzyme as well as the role of various redox centers in catalysis. We have investigated in detail four inhibitors of *A. vinelandii* and the results are summarized here. The influence of these inhibitors on the spectral properties of the enzyme are summarized in a later section.

Acetylene. In previous work, we determined that acetylene is a slow-binding, site-directed inhibitor of *A. vinelandii* and *B. japonicum* hydrogenase (Hyman and Arp, 1987). While still bound to the membranes, *A. vinelandii* hydrogenase can be obtained aerobically in an inactive, but activatable, state. This aerobically prepared, inactive form of hydrogenase was not sensitive to acetylene, but could be made acetylene sensitive by reduction (leading to activation) of the enzyme. The results indicated that acetylene inhibition requires catalytically competent enzyme (Hyman et al., 1988). In continued studies of the mechanism of acetylene inhibition, we have investigated the ability of n-terminal alkynes to inhibit hydrogenase. Although inhibition was observed with propyne, the rate of inhibition was that expected from the level of acetylene which contaminated the propyne. Therefore, no substantial rate of inhibition could be associated with propyne. Likewise, n-butyne did not lead to any inhibition of hydrogenase activity. Ethylene was also not an inhibitor of hydrogenase. Thus, the inhibition was specific for acetylene. Our current model for the inhibitory effect of acetylene is that the binding of acetylene is associated with the loss of a proton from acetylene and the formation of a Ni acetylide. Reversal of the inhibition (which requires an overnight incubation) would therefore require abstraction of a proton from the medium and reformation of the C-H bond in acetylene. As a first test of this model, we have determined (by GC analysis and a chemical test) that acetylene is released from the enzyme during the recovery phase (e.g. no chemical transformation of the acetylene has occurred). Further tests of this model are described in the Experimental Plan section of the grant proposal.

Attempts to induce a covalent attachment of the acetylene at the active site (by treatment with bromine to initiate a radical formation in the acetylene) were unsuccessful. Acetylene is competitive with H_2 for binding to hydrogenase. Therefore, we asked if acetylene could also protect the enzyme from O_2 inactivation, as does H_2 (see below). When the enzyme was incubated in the presence of acetylene alone, then the acetylene was removed and the enzyme incubated in the presence of H_2, full activity was recovered. When the enzyme was incubated in the presence of acetylene, then O_2, then exposed to recovery conditions, full activity was again restored after several hours. Thus, acetylene, like H_2, can protect the enzyme from O_2 inactivation.

Dioxygen. The effects of O_2 on hydrogenases are known to be complex. However, we have investigated these effects on the hydrogenase isolated from *A. vinelandii* and have placed

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many of the observations on a quantitative and rational basis. Basically, the effects of O_2 on active hydrogenase can be separated into two categories. First, O_2 is a rapid-equilibrium, reversible inhibitor of both the H_2 oxidation activity and the exchange activity of the enzyme. The inhibition was noncompetitive versus the electron acceptor methylene blue and uncompetitive versus the substrate, H_2. An inhibition constant of 5.5 uM was determined for the purified enzyme. The second effect of O_2 is that of an irreversible inactivation of the H_2 oxidation and exchange activities of *A. vinelandii* hydrogenase. This slower inactivation followed a first order process and gave a half-life of 5.9 minutes for purified enzyme. Surprisingly, the activity did not decay to zero; rather, a residual activity of about 10% of the original activity was obtained. Even after incubation of the enzyme in air for 24 hours, the activity remained at 10%. For membrane-associated enzyme, the half-life for inactivation was longer (46 min) and the residual activity was considerably higher (60%).

Various reagents were investigated for their ability to protect hydrogenase from inactivation by O_2. The only reagent which could do so was H_2. The protection by H_2 was concentration dependent but saturated with as little as 0.2 kPa H_2 in the gas phase. CO could not protect the enzyme from inactivation by O_2 nor did it prevent H_2 from protecting the enzyme. We propose that H_2 and O_2 are simultaneously bound to the enzyme in a "stalemate" such that O_2 cannot carry out the reaction needed to inactivate the enzyme because of the presence of H_2 and H_2 cannot be activated either to allow exchange or the oxidation of H_2 (e.g. with reduction of O_2). This state of the enzyme ($E^{H2,O2}$) has proven useful in subsequent investigations. (Seefeldt and Arp, 1989)

Aerobic purification of *A. vinelandii* hydrogenase. Most Ni and Fe containing hydrogenases are purified in an inactive state in the presence of air. Activation requires incubation under anaerobic conditions in the presence of a reductant. We have previously purified the hydrogenase from *A. vinelandii* in the absence of O_2 under reducing conditions (2 mM dithionite). However, given the apparent convergence of many properties of the NiFe hydrogenases over the past few years, it was of interest to determine if the *A. vinelandii* hydrogenase could also be purified under aerobic conditions. We have worked out a purification protocol based on the anaerobic purification, but with slight modifications. The modifications are required because the inactive, but activatable, enzyme under air is not stable under conditions where the active enzyme under reducing conditions is stable. For example, the activatable form loses the ability to be activated when incubated at pH 5.0 overnight. Therefore, the overnight dialysis step was carried out at pH 7.4. With the modified protocol, we are able to purify to homogeneity an inactive form of the enzyme under air. We have examined a number of activators; H_2 and dithionite are the most effective. The ability of the enzyme to be activated is not affected by acetylene, indicating that acetylene does not bind to this oxidized, inactive form of the enzyme. On the other hand, cyanide treatment leads to the loss of ability to activate the enzyme, indicating that cyanide can bind to this form of the enzyme. This is consistent with the mechanism of cyanide inactivation (see below). Once activated, the enzyme behaves essentially as the anaerobically purified enzyme with similar sensitivities to inhibitors and similar kinetic constants (K_m's for H_2 and electron acceptors).

The results indicate a similarity to other NiFe hydrogenases. Nonetheless, we do not intend to routinely purify enzyme under aerobic conditions. First and foremost, the yields are only 10% of those obtained in the anaerobic purification, even with the aforementioned modifications. Second, the enzyme preparations always contain mixtures of active and inactive enzyme. As a result, experiments are very dependent upon the history of the enzyme. Furthermore, the specific activities we obtain are not consistent and have not yet reached those obtained with the anaerobic purification. (Sun Jin-hua and Arp, Manuscript in preparation).

Cyanide. Cyanide serves as a strong ligand to most transition metals, and therefore has been used an an inhibitor of many metalloproteins. Nonetheless, there have been no extensive studies of the effects of cyanide on hydrogenases. In preliminary studies, it was apparent that cyanide has virtually no effect on active *A. vinelandii* hydrogenase. However, the characterization of an oxidized, inhibited state of the enzyme with H_2 and O_2 ($E^{H2,O2}$) provided a new avenue for this investigation. When this form of the enzyme was incubated in the presence of KCN, an irreversible inacti-

vation of the enzyme occurred. This inactivation was time- and KCN concentration dependent. The rate of inactivation decreased with decreasing pH. Whether this reflected a requirement for CN^- rather than HCN or reflected the dependence of the enzyme on the pH was not determined. When the inactivation was carried out with ^{14}C-cyanide, label remained associated with the enzyme following passage of the enzyme through a desalting column. The results have established conditions for binding yet another ligand to *A. vinelandii* hydrogenase. (Seefeldt and Arp, 1989)

NO. Nitric oxide is a potent inhibitor of several iron-sulfur containing enzymes. NO binds to iron sulfur centers, giving rise to Fe-S-NO complexes. NO has been shown to be an inhibitor of a number of hydrogenases, though no thorough kinetic investigations of the mechanism of NO binding to hydrogenase had been carried out. We investigated the ability of NO to affect the activity and activation of the soluble hydrogenase from *Alcaligenes eutrophus*. This enzyme is similar to that from *A. vinelandii* in that it contains Ni and Fe in two similar subunits. However, this enzyme consists of two additional subunits which contain flavin and additional FeS centers and provide the capacity to couple H_2 oxidation to NAD^+ reduction. With active enzyme, NO (8-150 nM) inhibited H_2 oxidation in a time and NO concentration dependent manner. The inhibition was not competitive with either H_2 or NAD^+. The inhibition was partially reversible upon removal of the NO. The diaphorase activity of the enzyme was not affected by NO (200 uM). NO also inhibited the ability of the enzyme to be activated. We interpreted these results in terms of variable binding of NO to the FeS centers of this enzyme. (Hyman and Arp, 1988).

The effects of NO on *A. vinelandii* hydrogenase were also investigated and found to be complex. NO caused a reversible inhibition of H_2 oxidation by membrane-associated hydrogenase that was not affected by the presence of H_2. During the 5 min time course of a typical assay, the inhibition could be completely reversed by adding Fe^{2+}-EDTA (to bind all the NO in a tight complex) to the assay cuvette (Fig. 1). However, when incubated with higher concentrations of NO under non-turnover conditions, a time-dependent, irreversible inactivation occurred (Fig. 2). Although all three activities (isotope exchange, H_2 oxidation, H_2 evolution) were inactivated, the rate of inactivation was slower for exchange ($t_{1/2}$ = 60 min) than for the other two reactions ($t_{1/2}$ = 21 and 26 min, respectively). In contrast to the results with membrane-associated hydrogenase, purified hydrogenase was not reversibly inhibited by NO during turnover. Rather, a time-dependent, irreversible inactivation of activity was observed. As with membrane-associated enzyme, a time-dependent inactivation also occurred under non-turnover conditions. Again, this non-turnover inactivation took place at higher concentrations of NO. The inability of H_2 to influence the binding of NO suggests that NO and H_2 do not share the same binding site. This is similar to the results with O_2; NO is known to interact with O_2-binding proteins as an analog of O_2. Furthermore, the differences between turnover and non-turnover conditions suggest that the redox state of the clusters influences the effects of NO. (Hyman and Arp, 1990; submitted).

Electron Paramagnetic Resonance (EPR) Investigations of *A. vinelandii* Hydrogenase.

In collaboration with Gerrard Jensen and Philip Stephens at the University of Southern California, we have carried out an investigation of several states of *A. vinelandii* hydrogenase as influenced by various inhibitors and the substrate H_2. The spectrum of the enzyme "as isolated" (i.e. in the presence of dithionite) reveals a complex g=1.94 type of spectrum typical of a [4Fe-4S] cluster interacting with another paramagnetic (most likely, another [4Fe-4S] cluster in this case) (Fig. 3). This spectrum is like that of *B. japonicum* as isolated and similar to that of the particulate hydrogenase from *Alcaligenes eutrophus* when reduced. The spectrum is unlike that of *Desulfovibrio gigas* NiFe hydrogenase in the reduced state where no signals attributable to FeS centers are present at g<2. The basic features of this spectrum are unaltered by addition of CO, C_2H_2 or H_2 to the sample, despite the fact that all three of these gases have been shown kinetically to interact with this form of the enzyme. Because all three of these compounds bind to the H_2 binding site of *A.*

vinelandii hydrogenase, their lack of influence on the spectrum suggests that this signal does not arise from the site of H_2 interaction.

Oxidation of the enzyme with O_2 results in a rapid loss of the g=1.94 signal and formation of a new signal with a major feature centered at g=2.02 and satellite features at g=2.06 and 1.97 (Fig. 4). During a time course of O_2 inactivation, the major feature decreases in intensity, the satellite line at 2.06 disappears and the satellite line at 1.97 increases in intensity. Re-reduction of the sample with dithionite (which does not result in reactivation) results in reformation of the g=1.94 signal of the "as isolated" enzyme (Fig. 6). In addition, signals at 2.09, 2.20 and 2.22 are apparent and, by analogy to other hydrogenases, are assigned to Ni. Thus, O_2 inactivation does not alter the iron sulfur clusters(s) which give rise to the g=1.94 signal, but does appear to influence the Ni center. The g=2.02 feature of the oxidized enzyme is tentatively assigned to a [3Fe-4S] cluster. It appears that this center also retains the ability to undergo reduction following O_2 inactivation.

When we examined the $E^{H2,O2}$ state of the enzyme, we found that the enzyme was in an oxidized state as indicated by the loss of the g=1.94 signal and the appearance of the g=2.02 signal (Fig. 5). However, the satellite lines were lost and the 2.02 signal was notably broadened. Thus, as suggested by the kinetic results, H_2 influenced the oxygenated state of the enzyme. When the enzyme was re-reduced, the EPR signal returned to that of the "as isolated" enzyme. When enzyme was incubated in the presence of C_2H_2 and O_2, the EPR spectrum was similar to that of $E^{H2,O2}$. This further developed the concept that H_2 and C_2H_2 interact with the enzyme in similar manners.

Cyanide influenced the EPR spectrum of $E^{H2,O2}$ primarily by causing a sharpening of the 2.02 signal. When reduced hydrogenase was treated with NO (100% for 5 min), the signal was altered to that typical of the Fe-S-NO complex described for other proteins. (Jensen, Seefeldt, Arp, Stephens, 1990, Manuscript in preparation)

UV-Vis Spectral Investigations of *A. vinelandii* Hydrogenase

Previous investigations of the UV-Vis spectra of hydrogenases have been limited to comparisons of "oxidized" and "reduced". We have undertaken an extensive investigation of the UV-Vis spectral properties of *A. vinelandii* hydrogenase. This study was facilitated by the well-characterized inhibitors made available by our studies. The reduced, "as isolated" enzyme reveals a spectrum typical of FeS proteins (Fig. 7). The spectrum exhibits a broad absorption envelop from the edge of the aromatic amino acid absorption to about 600 nm. This absorption increases in the $E^{H2,O2}$ form of the enzyme (Fig. 8A). Difference spectra ($E^{H2,O2}$ - E^{act}) revealed absorption maxima at 435 and 325 nm. Again, this is typical of oxidized-reduced spectra of iron sulfur clusters. The difference spectrum of aerobically-purified-hydrogenase minus activated aerobically-purified-hydrogenase revealed a similar spectrum.

When hydrogenase was oxidized (and inactivated) by treatment with O_2, the 435 nm peak was broadened considerably and a new peak was observed at 315 nm (Fig. 8B). Re-reduction of the enzyme resulted in decreases in the 435 and 325 nm peaks, but the 315 nm peak was not altered by treatment with reductant. The 315 nm peak, therefore, appears to be correlated with the irreversible inactivation by O_2.

When reduced enzyme was treated with H_2, the spectrum was identical to that of enzyme treated with dithionite (followed by removal of the dithionite under an Argon atmosphere) (Fig. 7). In contrast, treatment of reduced enzyme with acetylene resulted in a time-dependent appearance of a new absorption peak with a maximum at 492 nm (Fig. 9). This absorption was not likely to be due to oxidation of the FeS centers for two reasons. First, the absorption was nearly 60 nm shifted from that of the oxidized enzyme and of much less intensity. Second, when the acetylene-inhibited enzyme was treated with O_2, the absorptions typical of the oxidized FeS centers appeared. Furthermore, this peak did not appear when the enzyme was treated with acetylene in the presence of H_2 (which prevents the inhibition by C_2H_2). We are tentatively assigning this absorp-

tion to a Ni:acetylide which forms upon inhibition with acetylene. If so, then this would constitute the first identification of a contribution of Ni to a visible absorption spectrum of a hydrogenase.

When the $E^{H2,O2}$ is treated with cyanide, a substantial change in the absorption spectrum occurs, indicative of cyanide binding to the enzyme. In contrast, when E^{H2} is treated with cyanide, no change in the absorption spectrum occurs. This is consistent with the kinetic results. (Sun and Arp, Manuscript in preparation)

Collaborative Projects

Inhibition of CO dehydrogenase from *Rhodospirillum rubrum* with NO and COS. Because of our interest in ligand interactions with metalloenzymes, we were interested to determine to what extent our observations with NiFe hydrogenase would extend to another NiFe enzyme, namely, CO dehydrogenase (CO-DH. In a collaborative effort with Scott Ensign and Paul Ludden (University of Wisconsin, Madison) we first examined the ability of acetylene to inhibit CO-DH. No inhibition was observed. We then examined the ability of NO to inhibit CO-DH. NO proved to be a potent inhibitor of CO-DH. It inactivates this enzyme in a time and concentration dependent manner. NO-treated apo-CO-DH (which lacks Ni) cannot be activated by addition of Ni, in contrast to the apoenzyme which has not been treated with NO. (Hyman, Ensign, Arp, Ludden; Manuscript in preparation).

We also investigated another potential inhibitor of this enzyme, namely, carbonyl sulfide (COS). COS proved to be a reversible inhibitor of this enzyme and gave a kinetic pattern versus CO which was indicative of competitive inhibition. COS inhibition was uncompetitive versus the electron acceptor, methyl viologen. COS does not appear to be an alternative substrate for CO-DH, given that the dye-oxidized enzyme is not reduced by COS. Rather, COS appears to be acting as a dead-end inhibitor. The significance of this is that it represents the first description of a rapid-equilibrium inhibitor of CO-DH which is competitive with CO. (Hyman et al., 1989). In contrast, COS does not inhibit *A. vinelandii* hydrogenase. CO-DH is not capable of catalyzing the isotope exchange reaction catalyzed by *A. vinelandii* hydrogenase. Thus, these two NiFe enzymes exhibit distinct catalytic properties and inhibition patterns.

Immunological comparison of Fe-only and NiFe Hydrogenases. In a collaborative project with Kornel Kovacs and Len Mortenson (Univ. Georgia), we investigated the immunological cross-reactivity of 11 purified hydrogenases with 7 polyclonal antibodies raised against specific hydrogenases or hydrogenase subunits. The comparisons were carried out using Western blots to provide good sensitivity and discrimination of the subunits of multimeric hydrogenases. The results revealed substantial immunological cross-reactivity between the various NiFe hydrogenases investigated, even though the representative hydrogenases included very different physiological groups. Somewhat surprisingly, we also observed limited cross-reactivity between antisera prepared against an Fe-only hydrogenases and 4 NiFe hydrogenases and between antiserum prepared against a NiFe hydrogenase and an Fe-only hydrogenase. Whether this reflects a common ancestry of portions of these enzymes or the similarity of H_2 binding sites between these two classes of hydrogenases remains to be determined. (Kovacs et al., 1989)

Characterization of the structural genes of *Azotobacter chroococcum*. We determined the N-terminal amino acid sequences of the large and small subunits of *A. vinelandii* hydrogenase and *B. japonicum* hydrogenase. This information was used by Geoff Yates and coworkers (University of Sussex, Brighton, UK) to prepare an oligonucleotide for use in screening subclones of the genome which were known to contain HUP (hydrogen uptake) genes. In this way, the structural genes were identified and eventually sequenced. An analysis of the sequences was presented in the Literature Review section. (Yates et al, 1988; Ford et al., 1990).

Summary Statement

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

The results of this DOE-sponsored project have contributed to our understanding of the catalytic mechanism of *A. vinelandii* hydrogenase. A group of inhibitors have been characterized. These provide information about the different types of redox clusters involved in catalysis and the roles of each. The results will also be applicable to other NiFe hydrogenases. One group has already used acetylene in a study of three desulfovibrian hydrogenases and shown that only the NiFe hydrogenases are inhibited. The inhibitor studies are also being extended to other enzymes, both in this laboratory and in others. We have characterized a number of spectral properties of *A. vinelandii* hydrogenase. The EPR signals associated with this hydrogenase in the reduced state are reminiscent of other NiFe dimeric hydrogenases such as *A. eutrophus*, but distinctly different from others such as *D. gigas* and *Chromatium vinosum*. Thus, while the NiFe dimeric hydrogenases are now recognized as a large group of similar enzymes, there are differences in the spectral and catalytic properties which are not explained by their similar redox inventories, identical subunit structures, immunological cross reactivity and conserved sequences. The inhibitors we have characterized are also proving of value in the spectral characterizations. Surprisingly, we only see a significant EPR signal attributable to Ni after the enzyme has been inactivated with O_2 and then re-reduced (though not reactivated). No spectral perterbations (EPR or UV-Vis) of active enzyme can be attributed to binding of H_2, even though H_2 clearly binds to this form of the enzyme. Acetylene, which does not substantially perterb the EPR signal of active hydrogenase, does result in a new absorption envelope in the UV-Vis spectrum. Overall, the results of this project have revealed the complex interactions of the redox clusters in catalysis through studies of inhibitor mechanisms and spectral properties.

Manuscript, Meetings and Other Presentations of Research Results Resulting from This DOE-sponsored Project

I. Manuscripts

A. *In Press* or *Submitted* at the time of the last renewal request (7/15/87).

Hyman, M.R. and D.J. Arp (1987) Acetylene is an active-site-directed, slow-binding, reversible inhibitor of *Azotobacter vinelandii* hydrogenase. Biochemistry 26, 6447-6454. (DOE and USDA supported)

Seefeldt, L.C., L.C. McCollum, C.M. Doyle and D.J. Arp (1987) Immunological and molecular evidence for a membrane-bound, dimeric hydrogenase in *Rhodopseudomonas capsulata*. Biochim. Biophys. Acta 914, 299-303. (DOE supported)

Seefeldt, L.C. and D.J. Arp (1987) Redox-dependent subunit dissociation of *Azotobacter vinelandii* hydrogenase in the presence of sodium dodecyl sulfate. J. Biol. Chem. 262, 16816-16821. (DOE supported)

B. Submitted and Published since last renewal request (7/15/87)

Hyman, M.R. and D.J. Arp (1988) Reversible and irreversible effects of nitric oxide on the soluble hydrogenase from *Alcaligenes eutrophus* H16. Biochem J. 254, 469-475. (DOE and USDA supported)

Hyman, M.R. and D.J. Arp (1988) REVIEW: Acetylene Inhibition of metalloenzymes. Anal. Biochem. 173, 207-220. (DOE, USDA and NSF supported).

Yates, M.G., C.M. Ford, K.H. Tibelius, F. Campbell, D.J. Arp and L.C. Seefeldt (1988) Aspects of the physiology and genetics of the H_2-uptake hydrogenase of *Azotobacter chroococcum*. *in* Nitrogen Fixation: Hundred Years After, Proc 7th Int. Conf. N_2 Fix. (H. Bothe, F.J. deBruijn, W.E. Newton, eds.) Fischer, Stuttgart, pp. 263-269. (DOE supported).

Hyman, M.R., L.C. Seefeldt and D.J. Arp (1988) Aerobic, inactive forms of *Azotobacter vinelandii* hydrogenase: Activation kinetics and insensitivity to C_2H_2 inhibition. Biochim. Biophys. Acta 957, 91-96. (DOE supported)

Kovacs, K.L., L.C. Seefeldt, G.Tigyi, C.M. Doyle, L.E. Mortenson and D.J. Arp (1989) Immunological relationship among hydrogenases. J. Bacteriol. 171, 430-435. (DOE and NSF supported)

Seefeldt, L.C. and D.J. Arp (1989) Oxygen effects on the nickel- and iron-containing hydrogenase from *Azotobacter vinelandii.* Biochemistry 28, 1588-1596. (DOE supported)

Arp, D.J. (1989) Hydrogen-oxidizing bacteria: methods used in their investigation. *in* Modern Methods of Plant Analysis, New Series, Vol. 9, Gases in Plant and Microbial Cells (H.J. Linskens and J.F. Jackson, eds.) Springer-Verlag, Berlin, pp. 257-274 (DOE and NSF supported)

Seefeldt, L.C. and D.J. Arp (1989) Cyanide inactivation of hydrogenase from *Azotobacter vinelandii.* J. Bacteriol. 171, 3298-3303. (DOE supported)

Hyman, M.R., S.A. Ensign, D.J. Arp and P.W. Ludden (1989) Carbonyl sulfide inhibition of CO dehydrogenase from *Rhodospirillum rubrum.* Biochemistry 28, 6821-6826. (DOE supported)

C. In press or submitted

Ford, C.M., N. Garg, R.P. Garg, K.H. Tibelius, M.G. Yates, D.J. Arp and L.C. Seefeldt (1990) The identification, characterization, sequencing and mutagenisis of the genes (hupSL) encoding the small and large subunits of the H2-uptake hydrogenase of *Azotobacter chroococcum.* Molecular Microbiol. In press. (DOE supported).

Hyman, M.R. and D.J. Arp (1990) Kinetic analysis of the interaction of nitric oxide with the nickel-and iron-sulfur containing hydrogenase from *Azotobacter vinelandii.* Submitted to Biochim. Biophys. Acta. (DOE supported).

II. Meetings

Research results from this DOE-sponsored project were presented at the following scientific conferences:

Hydrogenase Meeting, Unicoi, Georgia; September 1988 (DJA, LCS, MRH)

6th International C_1 Symposium; Gottingen, West Germany; August, 1989 (DJA)

UCLA Symposium: Inorganic Chemistry and Molecular Biology Interface; Taos, New Mexico; February 1990 (MRH)

8th Int. Symposium N_2 Fixation; Knoxville, Tennessee; May 1990 (DJA, SJH)

III. Other

The results of this research were presented in several seminars and shared informally with colleagues at several institutions. Materials (e.g. antibodies and purified enzyme) were also frequently shared with colleagues at several institutions.

Figure 1
NO (5 uM) Reversibly Inhibits Membrane-associated Hydrogenase H_2 Oxidation Activity

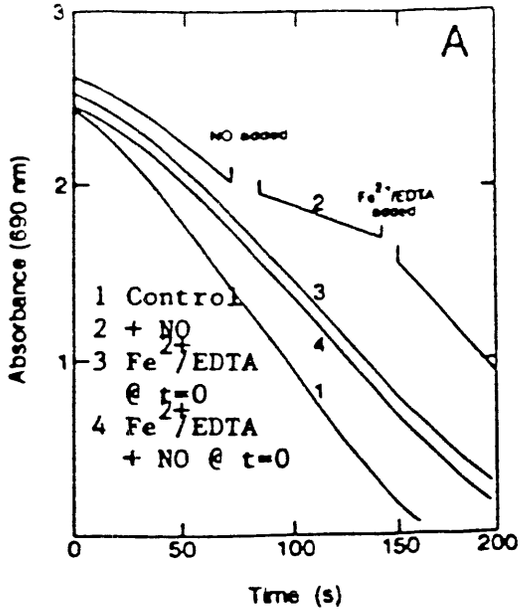


Figure 2
NO (250 uM) Irreversibly Inactivates Hydrogenase Activity

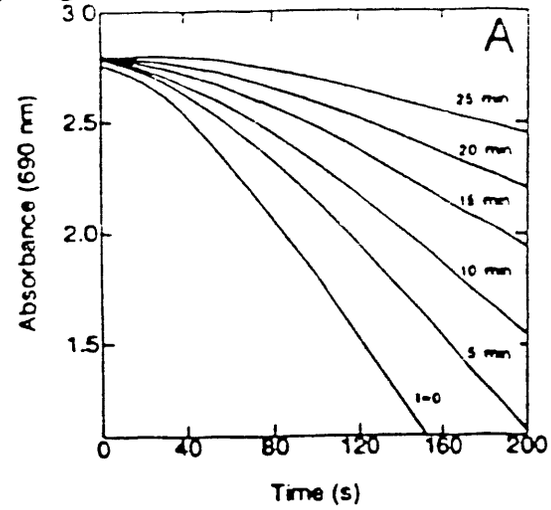


Fig. 2. EPR Spectra of NiFe Hydrogenases

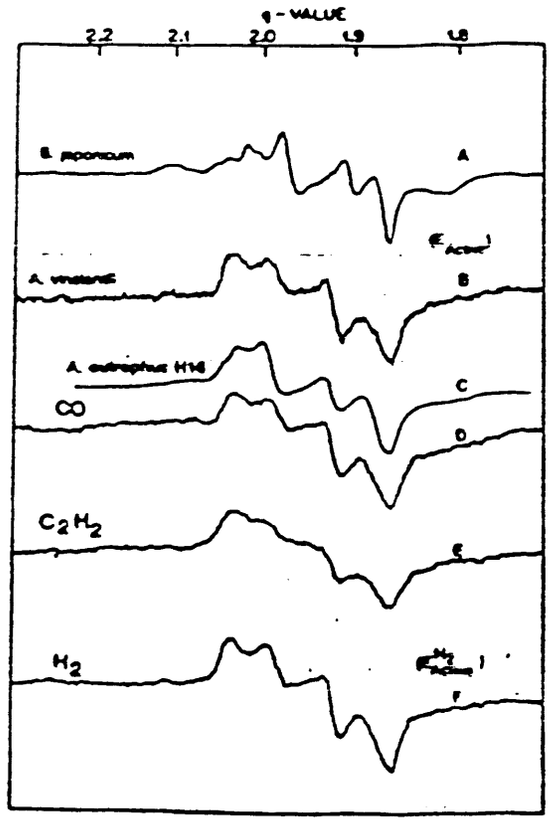


Fig. 3. EPR Spectra of A. vinelandii Hydrogenase during O_2 Inactivation

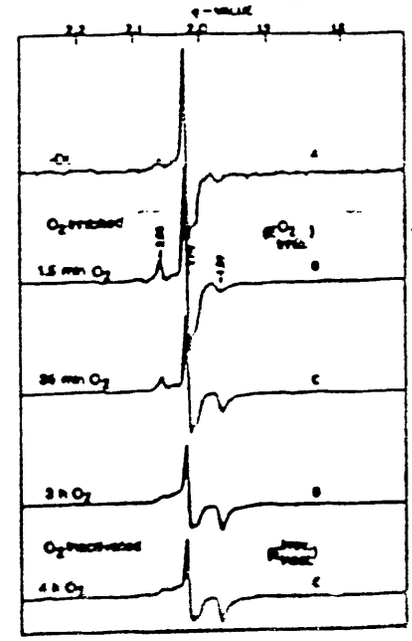
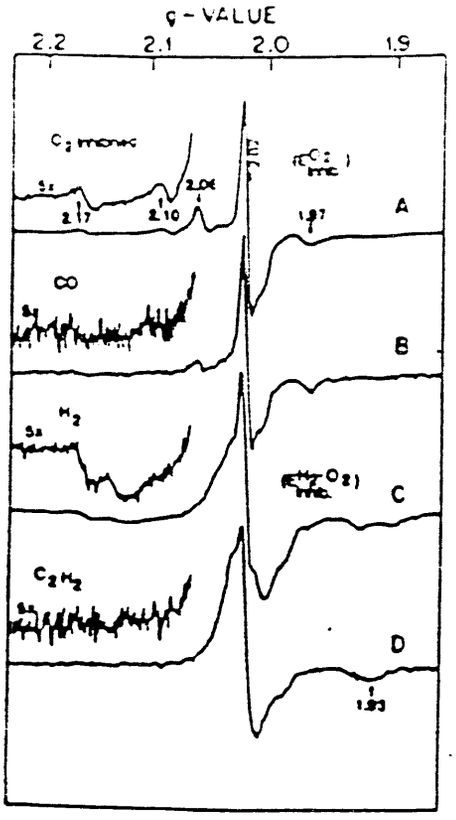


Figure 5

EPR SPECTRA OF O_2-INHIBITED

A. vinelandii HYDROGENASE TREATED WITH VARIOUS INHIBITORS



gure 6

EPR SPECTRA OF H$_2$-REDUCED O_2-INHIBITED AND O_2-INACTIVATED A. vinelandii HYDROGENASE

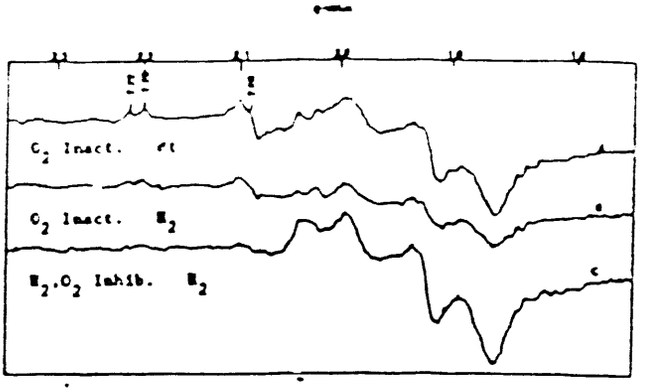


Figure 7

UV-VIS SPECTRUM OF

A. vinelandii HYDROGENASE

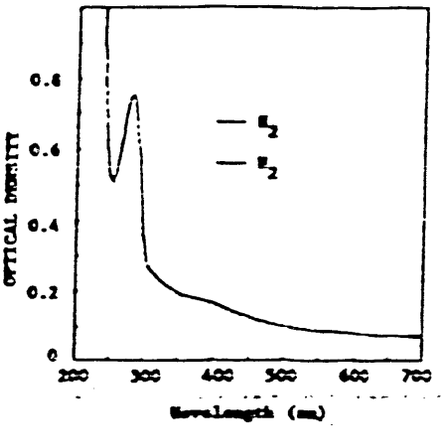


Figure 8

EFFECT OF O_2 ON

A. vinelandii HYDROGENASE SPECTRA

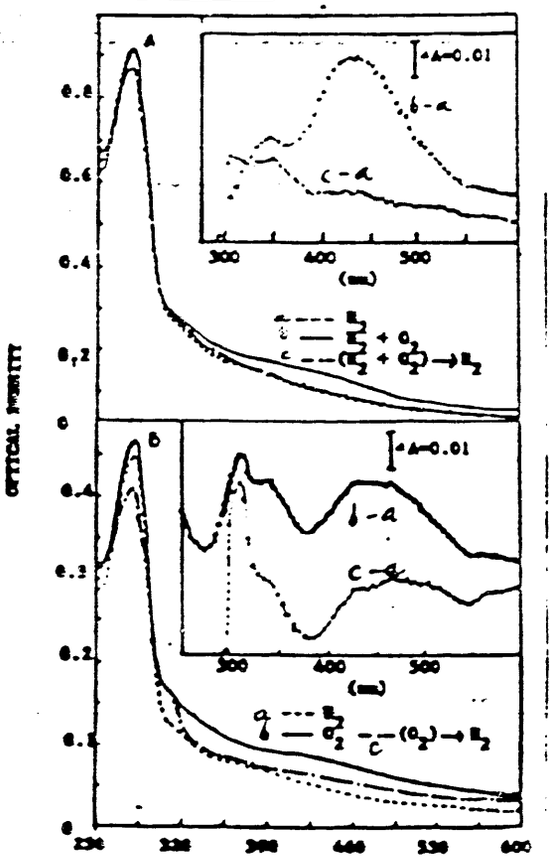
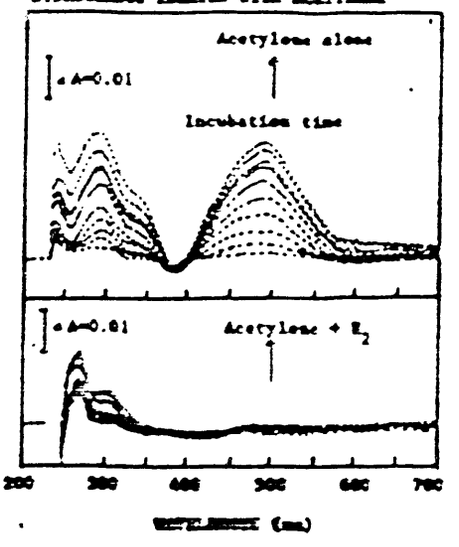


Figure 9

DIFFERENCE SPECTRA OF A. vinelandii

HYDROGENASE TREATED WITH ACETYLENE



END

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01/03/92