Chemical Evolution of a Nitrogenase Model. IV. Reduction of Isonitriles[1,2]

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Abstract: Catalysts composed of molybdate and a thiol component, *e.g.*, cysteine, duplicate functional properties of nitrogenase enzymes. Using $NaBH_4$ or $Na_2S_2O_4$ as reducing agents, molybdate–cysteine catalysts reduce isonitriles to CH_4, C_2H_4, C_2H_6, C_3H_6, and C_3H_8. The reaction is significantly stimulated by ATP and other phosphorylating agents and is competitively inhibited by carbon monoxide and nitrogen. The results reaffirm previous conclusions concerning the role of molybdenum in the binding and reduction of nitrogen and the alternate substrates of nitrogenase. For iron, the more abundant constituent of nitrogenase, no similar catalytic functions could be demonstrated in homogeneous Fe^{2+}–cysteine systems; although some reduction of isonitriles to hydrocarbons was observed, ATP caused a virtual loss of catalytic activity. However, iron salts were found to exert a cocatalyst effect in molybdate–cysteine systems, indicating that iron may function as an electron-transfer catalyst, accelerating the reduction of the molybdate–cysteine complex to the catalytically active reduced form.

Cell extracts, *e.g.*, of *Clostridium pasteurianum*, *Azotobacter chroococcum*, and *Mycobacterium flavum 301*, as well as purified nitrogenase (N_2-ase) holoenzyme from *Azotobacter vinelandii*, reduce isonitriles in the presence of $Na_2S_2O_4$ and an ATP-generating system to form hydrocarbons derived from the isonitrile carbon atom and primary amine from the R–N moiety of these substrates.[3–9] These reactions are of interest as examples of multielectron reductions catalyzed by N_2-ase. The conversion of the isonitrile carbon atom to CH_4, C_2H_4, C_2H_6, C_3H_6, and C_3H_8 requires 6, 8, 10, 12, and 14 electrons, respectively. Since the reduction of N_2 to $2NH_3$ involves the transfer of six electrons, isonitriles may serve as model substrates for biological nitrogen fixation. Although the reduction of isonitriles is known to be transition metal catalyzed,[10] and organometallic insertion reactions of isonitriles have been reported,[11] no systematic correlation of isonitrile reduction in model systems with the N_2-ase-catalyzed reduction has been described. In previous communications we have shown that isonitriles are reduced by homogeneous catalyst systems composed of molybdate and a thiol component to yield C_1–C_3 hydrocarbons.[12,13] In the present paper we report on the reduction of these substrates in greater detail.

Model Systems Employed. As for most of the previous experiments with acetylenes, L-(+)-cysteine (Cys) was chosen as the thiol component and employed either as a mixture with Na_2MoO_4 in aqueous borate buffered or unbuffered solution, or in the form of the binuclear Mo(V) complex,[14] $Na_2Mo_2O_4(Cys)_2 \cdot 5H_2O$, hereafter designated I. Mercaptoethanol and thioglycerol were also tried as cofactors but were subsequently eliminated from further consideration in view of their tendency to slowly decompose with ethylene and propylene formation, respectively, under the reaction conditions, causing an undesirable hydrocarbon background interfering with the product assays.

With I–$NaBH_4$, the concentration of hydrocarbons in the gas phase produced from CH_3NC as the substrate increases linearly with time during at least the first 100 min of incubation at 20–25° (Figure 1), provided that an excess of reducing agent is present. Initial rates, whenever quoted, were obtained during the first 40–60 min of incubation. Brief (*ca.* 5 min) induction periods are observed occasionally in systems with I, but not with MoO_4^{2-}–Cys. The optimal pH of the reduction of isonitriles by MoO_4^{2-}–Cys (1:1) lies between 5 and 11, rate maxima appearing at pH 6 and 10, with $NaBH_4$ as a reducing agent (Figure 2). With $Na_2S_2O_4$ as reductant the optimal pH is 10 (Figure 3). To obtain results comparable with the previous work with acetylenic substrates, all isonitrile reduction experiments were carried out at around pH 9.6 in borate-buffered solutions.

The Optimal MoO_4^{2-}/Cys Ratio. The optimal MoO_4^{2-}/Cys ratio to achieve maximum catalytic activity for isonitrile reduction was determined by studying the system MoO_4^{2-}–Cys with $NaBH_4$ and $Na_2S_2O_4$ as reductants, both in the absence and presence of ATP. In all cases maximum catalytic activity for the reduction of CH_3NC was observed at the MoO_4^{2-}/Cys ratio of 1:1. The results demonstrate the essentiality of Cys as a cofactor and justify the employment of I as the catalyst, as it contains molybdenum and cysteine

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(2) Communicated at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 14, 1971.

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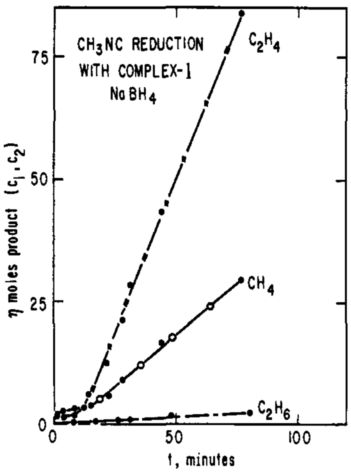

Figure 1. Catalytic reduction of CH_3NC with I-$NaBH_4$ in the absence of ATP. The figure shows the yields of CH_4 (—O—O—), C_2H_4 (–=–=–), and C_2H_6 (–––) during the first 80 min after the addition of $NaBH_4$ (reduction temperature 27°). Initial concentrations were: CH_3NC, 0.183 *M*, $NaBH_4$, 0.19 *M*. Concentration of I, 0.043 *M*. All reactions were performed in glass vials of 25-ml volume. The total solution volume was 3.5 ml in pH 9.6 borate buffer (0.2 *M*).

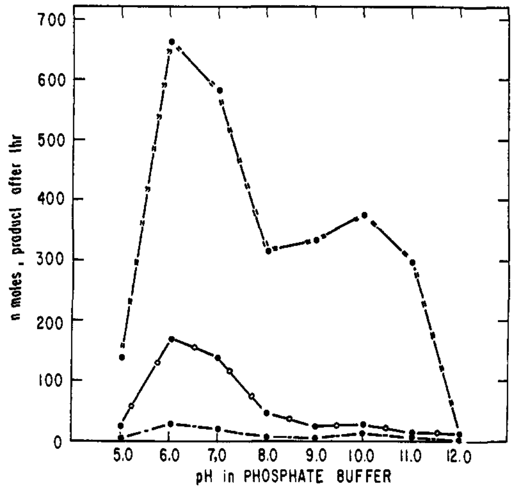

Figure 2. Catalytic reduction of CH_3NC in the system Na_2MoO_4–Cys (1:1 molar ratio), at various solution pH values, with $NaBH_4$ as reductant. Product yields of CH_4 (—O—O—), C_2H_4 (–=–=–), and C_2H_6 (–––). The pH was adjusted by phosphate buffers. Results quoted are after 1 hr of incubation at 27°. Initial concentrations were: $NaBH_4$, 0.19 *M*; CH_3NC, 0.183 *M*; $[Na_2MoO_4]$ = [Cys] = 0.107 *M*. The total volume of the reaction solution was 3.5 ml.

in the molar ratio of 1:1. The results of the systems studied are represented graphically in Figures 4 and 5. It may be seen that the catalytic activity of the systems is much higher with $NaBH_4$ than with $Na_2S_2O_4$ as reductant, as was also observed in the previous experiments with acetylenic substrates. The ATP effect upon the catalytic activity is also greater with $NaBH_4$ than $Na_2S_2O_4$, which is likewise a consequence of the lower efficiency of the latter reductant as compared to the former. Compared to acetylene, isonitriles are reduced at 10^{-3}–10^{-4} times slower rates by our model systems. Although isonitriles are also reduced more slowly than acetylene by N_2-ase, the rate differs only by a factor of 10^{-1}. The much slower rates observed

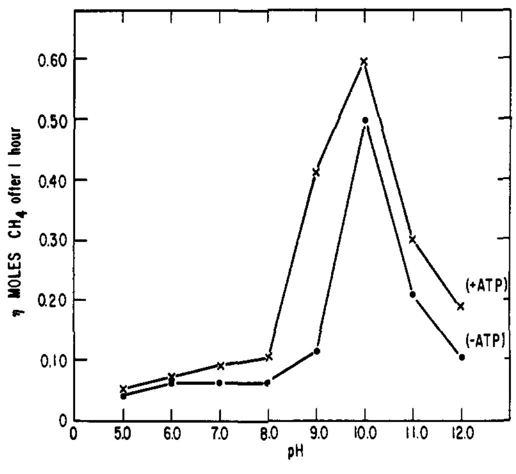

Figure 3. Catalytic reduction of *tert*-ButNC by Na_2MoO_4–Cys (1:1 molar ratio) at various solution pH values, with $Na_2S_2O_4$ as reducing agent. Product yield of CH_4 quoted after 60 min of incubation at 27° (yields of C_2 and C_3 hydrocarbons are too low under these conditions and are not included in the graph). Solution pH was adjusted with appropriate phosphate buffers (0.2 *M*). Initial concentrations were: $Na_2S_2O_4$, 0.155 *M*; *tert*-BuNC, 0.350 *M*; $[Na_2MoO_4]$ = [Cys] = 0.087 *M*. The total reaction volume was 4.3 ml.

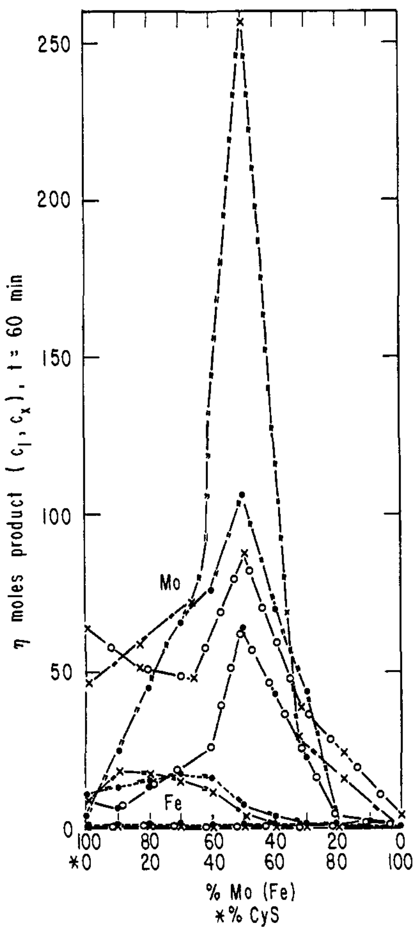

Figure 4. Catalytic activity in the systems Na_2MoO_4–Cys and Fe^{2+}–Cys (reducing agent, $NaBH_4$) in the reduction of CH_3NC. Products after 60 min of incubation: CH_4 (—O—O—), C_2H_4 (–=–=–) in the absence (●) and presence (×) of ATP. The sum of the molar concentrations of Na_2MoO_4 and of Cys is 0.214 *M* at each molar ratio, and is 0.086 *M* of Fe^{2+} (supplied as $FeSO_4 \cdot 7H_2O$) and Cys. Initial concentrations were: $NaBH_4$, 0.191 *M*; CH_3NC, 0.183 *M*; ATP, 0.171 *M*. The total reaction volume was 3.5 ml, in 0.2 *M* pH 9.6 borate buffer.

Table I. Initial Rates of Hydrocarbon Production from Isonitriles under Various Conditions

Catalyst	M	Substrate	M	CH_4 −ATP	CH_4 +ATP	C_2H_4 −ATP	C_2H_4 +ATP	C_2H_6 −ATP	C_2H_6 +ATP	C_3H_8 −ATP	C_3H_8 +ATP	C_3H_6 −ATP	C_3H_6 +ATP
I	0.0013	CH_3NC	0.183	0.034	0.095	0.035	0.150	0.0008	0.0055	*a*	*a*	*a*	*a*
	0.0026		0.183	0.041	0.104	0.058	0.165	0.0009	0.0064	*a*	*a*	*a*	*a*
	0.0051		0.183	0.049	0.147	0.078	0.182	0.0012	0.0074	*a*	*a*	*a*	*a*
	0.0106		0.183	0.051	0.189	0.091	0.233	0.0010	0.0072	*a*	*a*	*a*	*a*
	0.0212		0.183	0.061	0.226	0.098	0.284	0.0011	0.0101	*a*	*a*	*a*	*a*
	0.0428		0.183	0.068	0.243	0.129	0.347	0.0012	0.0083	*a*	*a*	*a*	*a*
	0.00015	$C_6H_{11}NC$	0.009	0.0068	0.008	0.003	0.004	0.0009	0.0009	0.0002	*a*	0.0003	*a*
	0.00030		0.0091	0.015	0.152	0.010	0.004	0.0034	0.0008	0.0003	*a*	0.0009	*a*
	0.00062		0.0091	0.063	0.110	0.040	0.006	0.054	0.0006	0.013	*a*	0.0036	*a*
	0.0013		0.0091	0.200	0.290	0.077	0.018	0.210	0.0007	0.010	*a*	0.0064	*a*
	0.0026		0.0091	0.323	0.375	0.116	0.110	0.290	0.0009	0.007	*a*	0.0070	*a*
	0.0051		0.0091	0.237	0.270	0.118	0.047	0.159	0.035	0.006	*a*	0.0037	*a*
	0.0110		0.0091	0.148	0.409	0.124	0.288	0.033	0.072	0.006	*a*	0.0009	*a*
	0.0220		0.0091	0.095	0.498	0.093	0.263	0.009	0.054	0.007	*a*	0.0005	*a*
	0.0428		0.0091	0.045	0.547	0.068	0.084	0.003	0.027	0.005	*a*	0.0004	*a*
	0.0428	*tert*-BuNC	0.008	0.016	0.235	0.077	0.007	0.002	0.0002	*a*	*a*	*a*	*a*
MoO_4^{2-}–Cys, 1:1	0.0125	CH_3NC	0.183	0.105	1.14	1.17	2.99	0.008	0.144	*a*	*a*	*a*	*a*
	0.0125	*tert*-BuNC	0.714	0.575	1.89	0.873	5.08	0.125	0.480	*a*	*a*	*a*	*a*
	0.0125	$C_8H_{14}N_2O$[b]	0.109	0.595	1.29	0.576	3.83	0.029	0.782	*a*	*a*	*a*	*a*

[a] Insufficient product yields for rate determination. [b] $C_8H_{14}N_2O$ is 3-*N*-morpholine-*n*-propyl isocyanide.

Initial rates, nmol/min

in the model studies suggest a lower affinity of the active-reduced Mo–Cys complex for isonitriles as compared to the active site of N_2-ase.

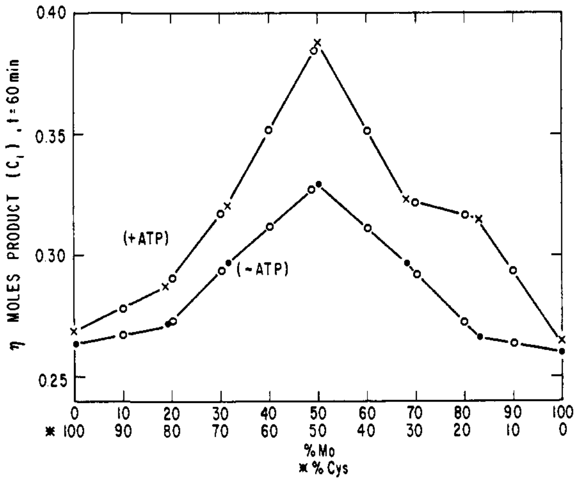

Figure 5. Catalytic activity in the system Na_2MoO_4–Cys with $Na_2S_2O_4$ as reducing agent, in the reduction of CH_3NC. The figure shows CH_4 produced after 120 min of incubation in the absence (—●—) and presence (—×—) of ATP at various molar ratios of Na_2MoO_4 and Cys. The sum of the molar concentrations of Na_2MoO_4 and Cys is 0.187 *M* at each molar ratio. Initial concentrations were: $Na_2S_2O_4$, 0.166 *M;* CH_3NC, 0.16 *M;* ATP, 0.15 *M*. The total volume of reaction solution was 4.0 ml in 0.2 *M* pH 9.6 borate buffer.

A compilation of initial rates of hydrocarbon production from four different isonitriles at different concentrations of catalyst (either I or MoO_4^{2-}–Cys, 1:1) and substrate, both in the absence and presence of ATP, is given in Table I. The initial rates of hydrocarbon production depend on the structure and concentration of the isonitrile and the catalyst concentration. With CH_3NC as the substrate at the initial concentration of 0.18 *M* the rates of CH_4, C_2H_4, and C_2H_6 formation increase with increasing concentration of I between 0.0013 and 0.04 *M*, both in the presence and absence of ATP. The dependence on catalyst concentration is not linear, indicating that I itself is not the actual catalyst. A plot of the rates of hydrocarbon formation as a function of $[I]^{1/2}$ shows regions of linearity (Figure 6) suggesting that the catalytically active species are monomeric. With the less volatile cyclohexyl isocyanide at the initial concentration of 0.01 *M*, the rates of CH_4, C_2H_4, C_2H_6, C_3H_6, and C_3H_8 production increase up to a concentration of I of 0.0025 *M* and decrease at higher concentrations of catalyst (Table I).

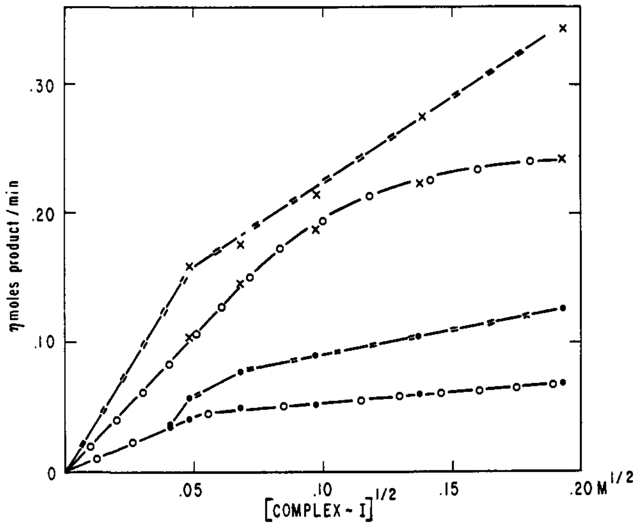

Figure 6. Rates of product formation from CH_3NC from I–$NaBH_4$, plotted against $[I]^{1/2}$ (rates determined after 60 min of incubation at 27°): CH_4 (—O—O—); C_2H_4 (‒ = ‒ = ‒), in the presence (×) and absence (●) of ATP. Initial concentrations were: CH_3NC, 0.183 *M;* $NaBH_4$, 0.183 *M;* ATP, 0.171 *M*, in a total volume of 3.5 ml of 0.2 *M* pH 9.6 borate buffer.

Effects of isonitrile substituents are much less pronounced than those in the case of acetylenic substrates. This is a clear indication of end-on binding of the isonitrile to molybdenum as opposed to side-on bonding of the acetylenes. In the absence of ATP, *tert*-ButNC

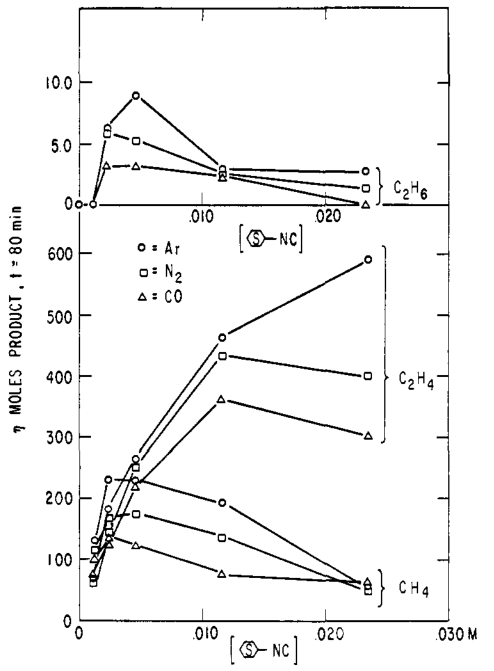

Figure 7. Inhibition of the reduction of cyclohexyl isocyanide by N_2 and CO relative to Ar (at 1 atm of partial pressure). The figure shows product yields of CH_4, C_2H_4, and C_2H_6 under argon (○), N_2 (□), and CO (△) as a function of isonitrile concentration. Initial concentrations were: $C_6H_{11}NC$, 0.0011–0.023 *M;* I, 0.043 *M;* $NaBH_4$, 0.191 *M;* in a total volume of 3.5 ml of pH 9.6 borate buffer (0.2 *M*); product yields after 80 min of incubation at 27°.

reduction by I–$NaBH_4$ affords high relative yields of ethylene (Table I). In the presence of ATP the rate of methane production increases more than tenfold, while the rate of ethylene formation drops to 0.1 the value in the absence of ATP.

Effect of ATP and Other Phosphates. The reduction of CH_3NC with I–$NaBH_4$ is significantly stimulated by ATP, less with ADP and AMP, and slightly inhibited by $Na_5P_3O_{10}$. The numerical data quoted in Table II are typical results obtained under identical reaction conditions and substrate concentrations. However, the degree of stimulation depends sensitively on the catalyst as well as the substrate concentration and the data listed in Table II reflect only the relative differences in the stimulating action of the mono-, di-, and triphosphates under one set of conditions chosen. As a general rule, ATP stimulates CH_4 and C_2H_6 production more than that of C_2H_4 at low substrate concentration (see, *e.g.*, Table I). The results in Table II were obtained at higher initial concentrations of CH_3NC, however, where ethylene stimulation is more significant.

Table II. Effect of Various Phosphates on Hydrocarbon Production from CH_3NC (Initial Concentration, 0.2 m*M*), with I–$NaBH_4$[a]

Addition	Isonitrile reduced, nmol	CH_4	C_2H_4	C_2H_6
ATP	392	44.0	172	1.8
ADP	262	42.0	105	5
AMP	296	54.7	119	1.5
$Na_5P_3O_{10}$	175	11.7	81.5	0
None	181	15.5	79.0	3.0

[a] Incubation time, 150 min.

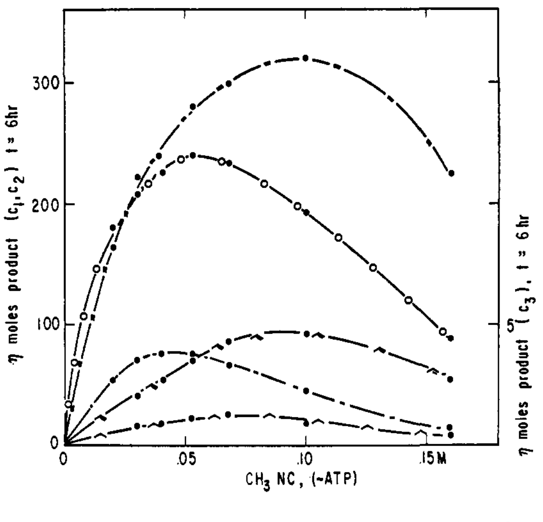

Figure 8. Dependence of product yields in the reduction of CH_3NC by I–$NaBH_4$ on the concentration of isonitrile, in the absence of ATP. Initial concentrations were: $NaBH_4$, 0.191 *M;* I, 0.043 *M,* in a total volume of 3.5 ml of 0.2 *M* pH 9.6 borate buffer. Yields recorded after 6 hr of incubation at 27°. The volume of glass vials was 25 ml: CH_4 (—o—o—); C_2H_6 (– — –); C_2H_4 (–=–=–); C_3H_6(-⋀̸-); C_3H_8(-⋀-).

Inhibition by N_2 and CO. The reduction of isonitriles by I–$NaBH_4$ is partially and reproducibly inhibited by N_2 and CO at 1 atm of partial pressure, relative to runs under argon. Nitrogen is a weaker inhibitor than CO, diminishing the hydrocarbon production on the average between 15 and 30%, while CO inhibits on the average between 35 and 50%. The degree of inhibition depends on the concentrations of both substrate and catalyst. As a representative example, Figure 7 shows the observed product yields from cyclohexylisonitrile under Ar, N_2, and CO as a function of the substrate concentration. The reproducibility of the experiments is excellent. It appears that *tert*-butyl isocyanide reduction is more subject to inhibition by N_2 or CO than is the reduction of CH_3NC. This may be a consequence of the difference in the K_m values. With methyl isocyanide but not with the other isonitriles studied, the production of methane was consistently enhanced by CO. It is possible that CO is reduced to CH_4 under certain conditions; we are presently investigating this reaction in greater detail.[14a]

Carbon monoxide (10% in Ar) inhibits CH_4 formation by N_2-ase from *Azotobacter chroococcum* by 90%, but increases the C_2H_4 production, while inhibiting C_2H_6 formation by only 50%.[3] Although CO is not reduced to CH_4 by N_2-ase, it is possible that it may undergo insertion reactions similar to isonitriles.

Yield Variation as a Function of Substrate Concentration. In the absence of ATP at a 0.04 *M* concentration of CH_3NC, CH_4 and C_2H_4 are produced in approximately equal amounts. The formation of CH_4 declines with increasing substrate concentration

[14a] Note Added in Proof. Experiments with $^{15}N_2$ at 1 atm of pressure have since been performed under conditions similar to the isonitrile reductions, indicating that molecular nitrogen is reduced to ammonia (G. N. Schrauzer, P. A. Doemeny, R. H. Frazier, and G. W. Kiefer, *J. Amer. Chem. Soc.*, in press).

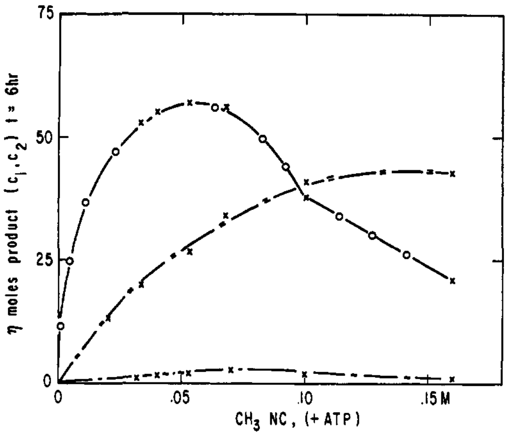

Figure 9. Dependence of product yields in the reduction of CH_3NC by I–$NaBH_4$ on the concentration of isonitrile, in the presence of ATP. Initial concentrations were: $NaBH_4$, 0.191 *M*; I, 0.043 *M*; ATP, 0.171 *M*, in a total volume of 3.5 ml of pH 9.6 borate buffer (0.2 *M*), after 6 hr of incubation at 27°. The volume of glass vials was 25 ml: CH_4 (—O—O—); C_2H_6(– — –); C_2H_4(–=–=–)

in favor of C_2H_4, C_3H_6, and C_3H_8. Inhibition of the reaction is observed at high concentrations of substrate (Figure 8). In the presence of substrate amounts of ATP, CH_4 production increases substantially, causing CH_4 to become the main product at low substrate concentrations (Figure 9). From the initial rates of product formation apparent K_m values were calculated according to Lineweaver and Burk for CH_3NC as the substrate. The K_m values obtained similarly for $(CH_3)_3$-CNC are higher, *i.e.*, 52 m*M* for CH_4 and 207 m*M* for C_2H_4 (in the presence of ATP), indicating diminished binding of the more highly substituted isonitrile substrate as compared to CH_3NC. A typical 1/*v vs.* 1/[s] plot is shown in Figure 10; observed K_m values are given in Table III.

Table III. Apparent K_m Values of Product Formation from CH_3NC by I–$NaBH_4$ Catalysts in the Absence and Presence of Substrate Amounts of ATP

	K_m, m*M*				
	CH_4	C_2H_6	C_2H_4	C_3H_8	C_3H_6
Without ATP	15.0	15.0	26.0	104	500
With ATP	8.9	625	125	500	625

Effect of D_2O. In the absence of ATP the initial rates of reduction of CH_3NC by I–$NaBH_4$ are slower in D_2O than in H_2O (Table IV). The rate of CD_4 formation in D_2O is 57%, that of C_2D_4 12%, and that of C_2D_6 56% of the rate of CH_4, C_2H_4, and C_2H_6 in H_2O, respectively. In the presence of substrate amounts of ATP the rates in D_2O increase substantially relative to those in H_2O, a fact which we attribute primarily to the slower rate of ATP hydrolysis in D_2O and a better allocation of electrons for substrate rather than D_3O^+ reduction.

Table IV. Initial Rates of Hydrocarbon Production (nmol/min, Incubation Time, 60 min), from CH_3NC with MoO_4^{2-}–Cys (1:1)–$NaBH_4$ in H_2O or D_2O in the Absence and Presence of Substrate Amounts of ATP[a]

	k_{CH_4}	k_{CD_4}	$k_{C_2H_4}$	$k_{C_2D_4}$	$k_{C_2H_6}$	$k_{C_2D_6}$
No ATP	0.346	0.199	23.3	2.73	0.086	0.048
With ATP	2.13	6.75	27.5	94.2	0.93	4.64

[a] $[CH_3NC]_{init}$ = 0.092 *M*; $[MoO_4^{2-}\text{–Cys}]$ = 0.0125 *M*.

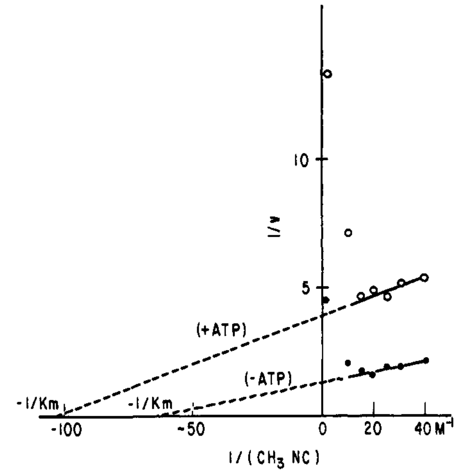

Figure 10. Lineweaver–Burk plot of the rate of methane production against the concentration of CH_3NC. Reaction vials of 25-ml volume contained a total solution volume of 3.5 ml, pH 9.6 borate buffer (0.2 *M*); $[Na_2MoO_4]$ = [Cys] = 0.107 *M*; initial concentrations of $NaBH_4$, 0.191 *M*, ATP, 0.171 *M*. Methane production was measured at intervals after $NaBH_4$ addition; initial velocity, *v*, expressed in moles/minute; substrate concentration, CH_3NC, expressed in millimoles/liter; K_m = 15.0 m*M* (without ATP), 8.9 m*M* (with ATP).

The Arrhenius energies of activation (E_a) of the formation of hydrocarbons from CH_3NC in H_2O and D_2O were obtained from measurements between 4 and 60° in the absence and presence of substrate amounts of ATP and are summarized in Table V. The E_a values of the formation of C_2H_4 and of C_2D_4 are identical within the error limits of the determination, while those of CD_4 and C_2D_6 are clearly greater than of CH_4 and C_2H_6, in the absence of ATP. The E_a values in the presence of ATP are lower for CH_4, CD_4, C_2H_4, and C_2D_4 (the data for C_2H_6 and C_2D_6 are not available due to the low product yields observed). The considerable diminution of the E_a of CD_4 formation in the presence of ATP indicates that ATP facilitates the reduction of the Mo–Cys complex to the catalytically active form, and that this process contributes significantly to the total energy of activation.

Table V. Arrhenius Activation Energies (E_a, kcal/mol) of Hydrocarbon Formation from CH_3NC with I–$NaBH_4$ in H_2O and D_2O in the Absence and Presence of ATP[a]

	Product[b]	CH_4	CD_4	C_2H_6	C_2D_6	C_2H_4	C_2D_4
No ATP	E_a (kcal/mol)	15.4	24.5	16.4	20.0	21.4	19.0
With ATP	E_a (kcal/mol)	12.1	9.1	*c*	*c*	17.3	16.0

[a] Error limits, ±0.5 kcal. [b] Formation of fully deuterated hydrocarbons in the experiments in D_2O was confirmed by mass spectrographic analysis of the gaseous reaction products. [c] Not determined due to low product yields.

Isonitrile Reduction in the System Fe^{2-}–Cysteine. Since N_2-ase contains iron and molybdenum the reduction of isonitriles could also occur on iron rather than molybdenum active sites, or on bimetallic centers. To establish the catalytic properties of iron under conditions where molybdenum is clearly capable of duplicating some of the key properties of N_2-ase, we have investigated the reduction of CH_3NC in the system Fe^{2+}–Cys with $NaBH_4$ as reductant. Although some reduction of the isonitrile was observed, ethylene was the main product and only traces of methane and ethane are formed. The Fe^{2+}–Cys system also loses activity within 20–30 min of incubation. The largest amounts of ethylene are produced at high Fe/Cys ratios. Under these conditions the system is heterogeneous, however. At the Fe^{2+}/Cys ratio of 1:1 and at higher levels of Cys, homogeneous solutions result whose activity is very low (Figure 4). Although there appears to be some stimulation by ATP at high Fe^{2+}/Cys ratios, ATP is actually inhibitory at the 1:1 ratio. With $Na_2S_2O_4$ as reducing agent, no catalytic activity was observed at the 1:1 Fe^{2+}/Cys ratio. It thus appears that homogeneous catalysts based on iron in the presence of Cys fail to duplicate N_2-ase reactions, in accord with previous conclusions reached with acetylene as the substrate. The addition of Fe^{2+} to MoO_4^{2-}–Cys catalysts causes a slight stimulation at low levels of Fe^{2+}, and inhibition of catalytic activity at concentrations greater than 25% of Fe^{2+} relative to molybdenum (Figure 11).

Metal Specificity. The reduction of isonitriles to hydrocarbons by transition metal ions or complexes is not molybdenum specific. Thus, active metals such as iron and zinc reduce CH_3NC to mainly methane in weakly acidic medium with concomitant production of methylammonium ion. However, heterogeneous reactions of this and similar types form a poor basis for model studies designed to achieve maximum convergence with N_2-ase reactions and were eliminated from further consideration.[15] In the presence of Cys a number of metal salts yield homogeneous solutions exhibiting catalytic activity for isonitrile reduction. With $NaBH_4$ as the reducing agent CH_3NC is reduced to C_1 and C_2 hydrocarbons by Co^{2+}, Pd^{2+}, Ru^{3+}, Rh^{3+}, and WO_4^{2-} in the presence of Cys at the molar ratio of 1:1 or 2:1. Although some of these systems are more active than those based on molybdenum, our experience to date indicates that CH_4 production is at best stimulated only slightly, and in some cases actually inhibited, in the presence of substrate amounts of ATP (Table VI). In addition, most of these catalysts yield products only during the first 40 min of incubation, while MoO_4^{2-}–Cys systems show substantial yield increments over prolonged periods. Of interest is the behavior of WO_4^{2-}–Cys catalysts relative to those of molybdenum. Although a greater activity is observed in the absence of ATP than with the latter, a substantial inhibition occurs in the presence of ATP (Table VI).

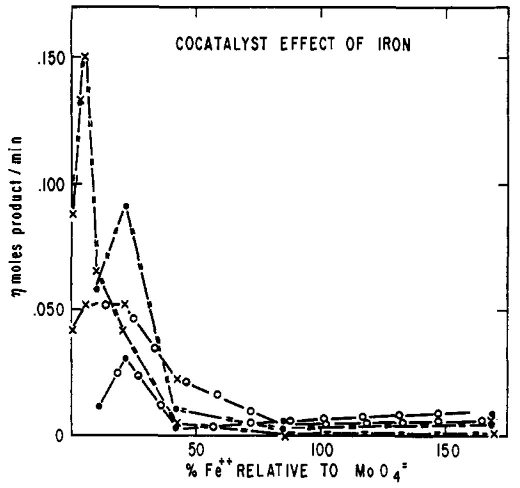

Figure 11. Catalytic reduction of *tert*-butyl isocyanide in the system Na_2MoO_4–Cys (molar ratio 1:1), with $NaBH_4$ as reductant, in the presence of iron (supplied as $FeSO_4 \cdot 7H_2O$), in the presence (×) and absence (●) of ATP. The figure shows yields of CH_4 —O—O—) and of ethylene (– = – = –) at different Fe^{2+}:MoO_4^{2-} molar ratios after 40 min of incubation at 27°. Initial concentrations were: Na_2MoO_4, 0.081 *M;* Cys, 0.081 *M;* Fe^{2+}, 0–0.276 *M;* *tert*-BuNC, 0.172 *M;* $NaBH_4$, 0.144 *M;* ATP, 0.129 *M*, in a total volume of 4.64 ml of pH 9.6 borate buffer (0.2 *M*).

Table VI. Relative Rates of Methane Production from CH_3NC in Systems Composed of Metal Salts and Cysteine[a]

Metal component	Rel rate of CH_4 production: −ATP	+ATP	ATP effect
MoO_4^{2-}	1.00	3.34	Stimulation
WO_4^{2-}	11.99	2.38	Inhibition
VO_4^{3-}	0.2	0.2	None
Pd^{2+}	1.65	1.19	Inhibition
Rh^{3+}	1.19	0.88	Inhibition
Ru^{3+}	0.307	0.57	Slight stimulation
Co^{2+}	7.61	6.71	Inhibition
Fe^{2+}	0.07	0.07	None

[a] At the molar ratio of 1:1, with $NaBH_4$ as reductant. Glass vials of 25-ml volume were 0.75 *M* in metal salt and Cys, 0.134 *M* in BH_4^- and 0.122 *M* in CH_3NC, in a total volume of 5.0 ml. In the experiments with ATP the solutions were 0.12 *M* in ATP under otherwise identical conditions.

Metal salts which proved inactive in the presence of Cys in isonitrile reduction include: $MnSO_4$, $OsCl_3$, $CrCl_3$, $IrCl_3$, $CuSO_4$, and $ZnCl_2$. With Na_3VO_4 a marginal amount of methane is produced from CH_3NC which remains unchanged in the presence of ATP, and which could be background (Table VI).

Discussion

The Mechanism of Isonitrile Reduction. The reduction of isonitriles to hydrocarbons by Mo-Cys catalysts is best described as the succession of one- or two-electron reduction steps with intermediate protonation, solvolysis, and isonitrile insertion reactions. The catalytically active reduced complex is presumably a Mo(IV)-Cys derivative possessing the possible structure A (with two vacant coordination sites in the cis

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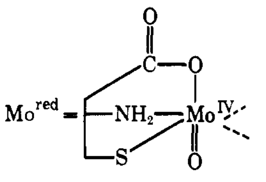
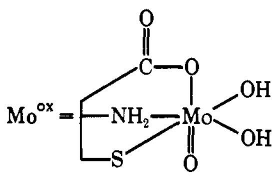
(15) Other examples of metal-reductant systems which duplicate N_2-ase reactions with isonitriles more remotely, if at all, were recently summarized by E. E. van Tamelen, *et al.*[16a] Our main objections against these nitrogenase models are that the majority of them operate in aprotic media, utilizing elements not present in N_2-ase in the absence of ligands simulating the protein binding site. Some of the systems described as nitrogenase models[17a] (*e.g.*, $MoCl_5$–Mg, $FeCl_3$–Mg, and $TiCl_3$–Mg) are not strictly homogeneous and/or operate under conditions far removed from those of the enzymatic reaction. These systems, furthermore, reduce cyclohexylisonitrile inefficiently, affording only small amounts of CH_4 and traces of C_2 hydrocarbons. In some cases cyclohexane was the only observed reaction product, indicating the destruction of the isonitrile by C–N bond cleavage, which as such does not occur in the biological system. Ideally, N_2-ase models should be based on the metals present in the enzyme and operate in homogeneous solution under physiological or near-physiological conditions obeying enzyme kinetics. None of the models in ref 16a, or in 16b, for which similar criticism may be raised, fulfills these requirements.

(16) (a) E. E. van Tamelen, H. Rudler, and C. Bjorklund, *J. Amer. Chem. Soc.*, **93**, 3526 (1971); (b) W. E. Newton, J. L. Corbin, P. W. Schneider, and W. A. Bulen, *ibid.*, **93**, 268 (1971).

(17) R. W. F. Hardy, R. C. Burns, and G. W. Parshall, *Advan. Chem. Ser.*, No. 100, 219 (1971).

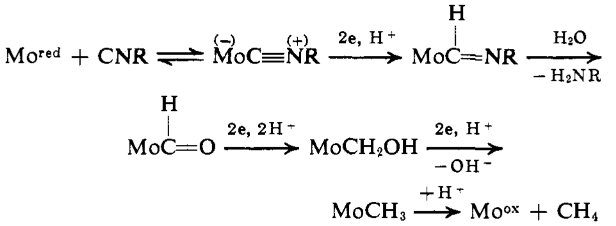


A



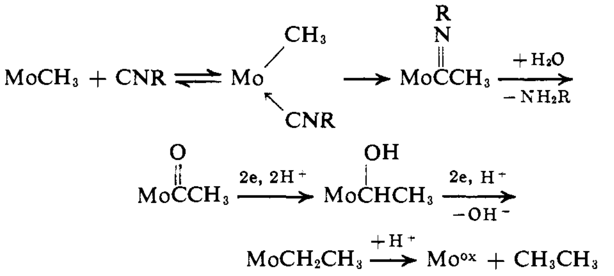
B

position) which hereafter will be designated $\mathrm{Mo^{red}}$. The oxidized Mo(VI) form of possible structure **B** will be similarly abbreviated $\mathrm{Mo^{ox}}$. The formation of methane, a six-electron reduction, may be represented accordingly by eq 1. Hydrolysis of the isonitrile carbon–nitrogen bond with formation of primary amine must take place after the reduction of the coordinated isonitrile by two electrons. It could not occur at later stages of the reduction, as this would give rise to the formation of a secondary amine, which would be incapable of undergoing C–N bond hydrolysis under the reaction conditions.



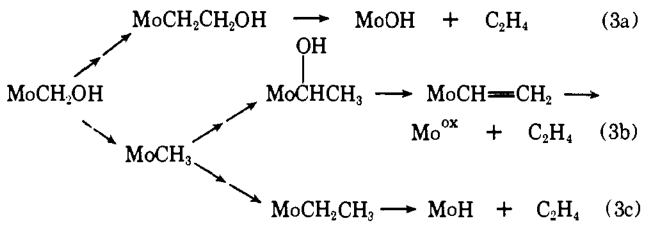
$$\mathrm{Mo^{red}} + \mathrm{CNR} \rightleftharpoons \overset{(-)}{\mathrm{Mo}}\mathrm{C}{\equiv}\overset{(+)}{\mathrm{N}}\mathrm{R} \xrightarrow{2e,\ H^+} \mathrm{Mo}\overset{H}{\mathrm{C}}{=}\mathrm{NR} \xrightarrow[-H_2NR]{H_2O}$$
$$\mathrm{Mo}\overset{H}{\mathrm{C}}{=}\mathrm{O} \xrightarrow{2e,\ 2H^+} \mathrm{MoCH_2OH} \xrightarrow[-OH^-]{2e,\ H^+}$$
$$\mathrm{MoCH_3} \xrightarrow{+H^+} \mathrm{Mo^{ox}} + \mathrm{CH_4} \quad (1)$$

The formation of ethane involves the insertion of one molecule of isonitrile into the Mo–C bond of an organomolybdenum intermediate. Most plausibly, the insertion step occurs prior to the hydrolysis of the Mo–$\mathrm{CH_3}$ bond, as is schematically represented in eq 2. Similar reduction and insertion mechanisms for isonitrile reduction by $\mathrm{N_2}$-ase have been proposed by Hardy, *et al.*,[17] and can now be linked specifically to a molybdenum active site of the enzyme. Organometallic insertion reactions of isonitriles have been observed with $\mathrm{Ni^{2+}}$, $\mathrm{Pd^{2+}}$, and $\mathrm{Pt^{2+}}$ organyl complexes.[11] The reactions reported in this paper represent, to our knowledge, the first examples of molybdenum-promoted isonitrile insertion and reduction reactions.



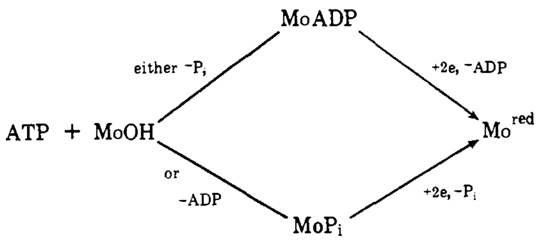
$$\mathrm{MoCH_3} + \mathrm{CNR} \rightleftharpoons \mathrm{Mo}\begin{smallmatrix}\mathrm{CH_3}\\ \mathrm{CNR}\end{smallmatrix} \longrightarrow \mathrm{Mo}\overset{\overset{R}{N}}{\overset{\|}{\mathrm{C}}}\mathrm{CH_3} \xrightarrow[-NH_2R]{+H_2O}$$
$$\mathrm{Mo}\overset{O}{\overset{\|}{\mathrm{C}}}\mathrm{CH_3} \xrightarrow{2e,\ 2H^+} \mathrm{Mo}\overset{OH}{\overset{|}{\mathrm{C}}}\mathrm{HCH_3} \xrightarrow[-OH^-]{2e,\ H^+}$$
$$\mathrm{MoCH_2CH_3} \xrightarrow{+H^+} \mathrm{Mo^{ox}} + \mathrm{CH_3CH_3} \quad (2)$$

The production of propane may be formulated in analogy to eq 2, involving isonitrile insertion into the Mo–C bond of a Mo–$\mathrm{C_2H_5}$ intermediate. Several pathways exist for the formation of ethylene which are summarized in eq 3 (isonitrile insertion and reduction steps are symbolized by double arrows). We consider the hydride elimination reaction 3c less likely in that it involves the same precursor required for the formation of ethane. It thus would be difficult to explain why significantly more ethylene than ethane is produced under certain conditions of reaction, *i.e.*, at high substrate levels in the absence of ATP. It also appears that ethylene production is favored under nonoptimal conditions of reduction. This could lead to an increase of the stationary concentration of $\mathrm{MoCH_2OH}$, which thus would become subject to isonitrile insertion and subsequent reduction to a β-hydroxyethylmolybdenum species. The latter could decompose with ethylene formation by way of a hydroxide ion elimination reaction (eq 3a). We favor this mechanism over the alternate pathway eq 3b, which would involve a methylmolybdenum intermediate also required for both methane and ethane formation. In the presence of ATP the accumulation of the hydroxymethyl intermediate would be prevented due to the more efficient transfer of electrons to molybdenum, leading to an increase in the methane production. A fourth mechanism of ethylene production was suggested by Kelly[6] on the basis of observations with $\mathrm{N_2}$-ase. This author noted the spontaneous decomposition of isonitrile solutions in the presence of buffer to produce acetylene. He therefore suggested ethylene to be the product of secondary reduction of acetylene by $\mathrm{N_2}$-ase. However, since we have consistently employed freshly prepared solutions of isonitriles which according to glpc analysis were essentially free of acetylene, this mechanism could not account for the formation of the major portion of ethylene in our reactions. It would also be difficult to explain the production of propylene on this basis, whose formation could be equally well accounted for by a mechanism analogous to eq 3a. In summary, it appears that ethylene is formed by a mechanism or by mechanisms involving a precursor or precursors of methylmolybdenum intermediates accumulating under nonoptimal conditions of reduction.



The Effect of ATP. The stimulation of the Mo–Cys catalysts by ATP and other phosphates was first demonstrated with acetylene as the substrate[18] and has been associated with the phosphorylation of a molybdenum-bound OH group, whose solvolysis is thus accelerated. This leads to a molybdenum(VI) species with a vacant coordination site which is evidently more rapidly reduced to the catalytically active form. In the absence of substrate, ATP stimulates the evolution of hydrogen by discharging protons from the reaction medium.[18]

The ATP effect is represented schematically in eq 4. The modes of interaction of ATP with a molybdenum-bound OH group are similar to the postulated ATP effect within the "two-site hypothesis" of $\mathrm{N_2}$-ase action



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[18] G. N. Schrauzer and P. A. Doemeny, *J. Amer. Chem. Soc.*, **93**, 1608 (1971).

Table VII. Characteristics of the Reactions of Nitrogenase and of the Model Systems with Isonitriles

Characteristic	N_2-ase	Model system
Products	CH_4, C_2H_6, C_2H_4, C_3H_6, C_3H_8	CH_4, C_2H_6, C_2H_4, C_3H_6, C_3H_8
Electrons/molecule	6–14	6–14
Estd activation energy, kcal/mol	14	15–21
Estd K_m (CH_3NC), m*M*	0.2–1	8–500
Rates rel to C_2H_2 reduction	1/4	1/10,000
Requirements	ATP, $Na_2S_2O_4$, N_2-ase	(ATP), $NaBH_4$, ($Na_2S_2O_4$), I
Effect of CO	Competitive inhibition	Competitive inhibition
Effect of N_2	Competitive inhibition	Competitive inhibition
Reaction in absence of substrate	H_2 evolution (ATP dependent)	H_2 evolution (ATP stimulated)
Effect of D_2O rel to H_2O	Reduction slower	Reduction slower in the absence of ATP, faster in the presence of ATP
Optimal pH	6–8	6–10 (with $NaBH_4$), 10 (with $Na_2S_2O_4$)

by Hardy, Knight, and Parshall.[19,20] They are also in accord with the previous work on the reduction of acetylenic substrates by molybdothiol catalysts.[18] Since one molecule of ATP is required for each two-electron transfer step, the consumption of excessive amounts of ATP in N_2-ase reactions is plausibly accounted for.

Effects of Inhibitors. The observed inhibiting effects of N_2 and CO demonstrate the interaction of these gases with the catalytically active Mo–Cys species, indicating a competition of N_2 and CO with isonitriles for coordination sites on molybdenum. Although CO is a stronger inhibitor than N_2, its effects are qualitatively similar to N_2.[14a]

Hydrocarbon Yield Ratios. Hydrocarbon yield ratios observed in the reduction of CH_3NC by N_2-ase vary with the origin and quality of the enzyme preparation. The rates of C_2 and C_3 hydrocarbon production are dependent on the substrate concentration and the rates of formation of respective hydrocarbon precursors. The following kinetic relations are obeyed[4]

$$d[C_2H_6]/dt = Kd[CH_4]/dt[CH_3NC] \qquad (5)$$

$$d[C_3H_6]/dt = K'd[C_2H_6]/dt[CH_3NC] \qquad (6)$$

For N_2-ase isolated from *Azotobacter vinelandii* at pH 7 in H_2O, $K = 6$, $K' = 2$, respectively.[4] From published[5] data on the reduction of CH_3NC by N_2-ase of *Azotobacter chroococcum*, K was calculated to be 2.0 (data for the calculation of K' are not available in this case). With I-$NaBH_4$ in the presence of substrate amounts of ATP, $K = 0.8 \pm 0.1$, $K' = 0.01$. The dependence of the relative yields of ethylene and ethane on the enzyme preparation suggests differences in the rates of reduction of the active site or the efficiency of ATP utilization, conceivably brought about by partial denaturation. Since N_2-ase yields consistently smaller amounts of C_2 and C_3 hydrocarbons than the model catalysts, and C_3 products are altogether absent if larger substrates, *e.g.*, C_2H_5NC, are employed, a partial steric obstruction of the active site is indicated. On the other hand, the available evidence indicates that the molybdenum-active site of N_2-ase is neither locally anhydrous nor in a strongly hydrophobic environment, since most of the substrates of N_2-ase are converted to the reduction products with solvent participation. In addition, the molybdenum-active site in N_2-ase must be accessible for interaction with ATP, a process which could likewise only occur in a basically hydrophilic environment.

In D_2O the rate of methane production from CH_3NC with N_2-ase from *A. chroococcum* is only 60% of that in H_2O, while there are 300 and 150% increases in the rate of ethylene and ethane formation.[3] With I-$NaBH_4$ in the absence of ATP the rate of methane formation is about 60% of that in H_2O, but, in contrast to the observations with N_2-ase, ethylene and ethane production is also inhibited. In the presence of substrate amounts of ATP, there is a 300–500% rate increase of the formation of C_1 and C_2 hydrocarbons in D_2O relative to H_2O due to the better allocation of electrons for substrate rather than H_3O^+ reduction and the slower rate of hydrolysis of ATP in D_2O.

Comparison with Nitrogenase. The observed values of K_m for the reduction of CH_3NC by N_2-ase are in the order of 0.2–1.0 m*M*.[21] The apparent K_m values for the reduction of the same substrate by I-$NaBH_4$ are larger by factors of at least 10 (Table IV), indicating a lower affinity of the active species in the model system for the isonitrile. This fact may be one of the reasons for the lower efficiency of the Mo-Cys models in the reduction of N_2-ase substrates and their diminished interaction with inhibitors such as CO. The binding of isonitriles, CO, or molecular nitrogen to transition metals is sensitively controlled by the degree of stabilization of the metal–ligand bond through d_π–p_π interactions, which ultimately depends on the energy of the d orbitals involved in this mode of bonding. Conceivably, the model systems presently in use do not supply sufficient d_π–p_π stabilization for the binding of N_2, CO, and isonitriles. For acetylenic substrates this deficiency is not as apparent, as acetylenes bind to transition metals with a lower degree of stabilization through d_π–p_π interactions.[22] The selection of ligands other than Cys capable of increasing the d_π–p_π bonding ability of molybdenum in the active reduced form will eventually lead to an improvement of the catalytic efficiency of the model systems. On the other hand, the qualitative convergence of the present model catalysts with N_2-ase is already far reaching (Table VII), and their ability to fix nitrogen to ammonia

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(19) R. W. F. Hardy and E. Knight, Jr., *Bacteriol. Proc.*, 112 (1967).

(20) G. W. Parshall, *J. Amer. Chem. Soc.*, **89**, 1822 (1967).

(21) See ref 5, 8, and 17.

(22) K. G. Caulton, R. L. DeKock, and R. F. Fenske, *J. Amer. Chem. Soc.*, **92**, 515 (1970).

at room temperature and normal pressure in the presence of catalytic amounts of iron has since been confirmed with $^{15}N_2$ by us as well as independently by Hill and Richards.[23] The observed inhibition of isonitrile reduction by molecular nitrogen in the absence of iron cocatalysts demonstrates conclusively that molybdenum possesses all the necessary properties for substrate binding and reduction. No similar function can be attributed to iron, which in the model systems is inactive or essentially inactive. The iron present in N_2-ase thus must be mainly involved in the catalysis of electron transport from the external reducing agent to the molybdenum active site. For this process to be efficient at least one iron atom of the electron transport system must be in close vicinity to the molybdenum active site. Thus, in the final analysis, our views deduced from the evidence accumulated in the model systems converge with the principal features of the "two-site hypothesis" of N_2-ase function, as long as the initial site of substrate binding and reduction is identified with molybdenum rather than iron.

Experimental Section

Reagents and Chemicals. Sodium borohydride (Ventron Corp.), L-(+)-cysteine hydrochloride (Nutritional Biochemicals Laboratories), and sodium molybdate (Baker AR grade) were used without further purification. Standard sodium borate buffer solutions were prepared from analytical grade chemicals in doubly distilled, deionized water. Methyl isocyanide was synthesized according to J. Casanova, *et al.*,[24] and was stored in glass ampoules immersed in liquid N_2. Cyclohexyl isocyanide, *tert*-butyl isocyanide, and morpholine-*N*-*n*-propyl isocyanide [$O(C_2H_4)_2N(CH_2)_3NC$] were synthesized by the method of,[25] or were gifts from, Dr. Ivar Ugi. The isonitriles were either added as such, or in the form of a freshly prepared aqueous solution of known concentration, to the reaction solutions. The Mo(V) complex of cysteine (I) was prepared according to Kay and Mitchell.[14] Cylinder N_2 was 99.998%, Ar, 99.995%, both from National Cylinder Gas. Carbon monoxide, CP (Matheson), was 99.9%. The gases were passed through alkaline pyrogallol solution and water before entering the reaction vessels.

Standard Gas Chromatographic Technique. All hydrocarbons were determined by glpc in the vapor phase using either a F & M Model 700 chromatograph or a Varian Series 1200 instrument, both equipped with a flame ionization detector. The gas samples were injected into a 6-ft Durapak, phenylisocyanate–Porasil C column made of $^1/_8$-in. copper tubing, at room temperature (27°), with He as carrier, at a flow rate of 10 ml/min. The retention times of the C_1, C_2, and C_3 hydrocarbons were determined by injecting standard samples of these gases. Calibration curves for concentration determinations were obtained by injecting measured volumes of methane, ethylene, and ethane accurately diluted with argon. The combined standard error of sample injection and peak height determination was determined to $\pm 1.5\%$ on the basis of ten measurements under identical conditions.

Catalytic Isonitrile Reduction in the System MoO_4^{2-}–Cys. Stock solutions of 0.25 *M* Na_2MoO_4 and 0.25 *M* Cys in pH 9.6 borate buffer (0.2 *M*) were prepared and stored in serum-capped flasks under pure argon. Varying known amounts of the molybdate and Cys solutions were added to 25-ml glass vials fitted with rubber septum and plastic caps, maintaining the sum of the volumes of the two solutions in each vial at 3.0 ml. The MoO_4^{2-}–Cys mixtures were purged with argon for 15 min. After this, the isonitrile was added by means of a micro-syringe. At $t = 0$, 0.5 ml of freshly prepared 1.33 *M* $NaBH_4$ solution was injected. The course of reduction was followed at 27° usually by the periodic withdrawal of 0.1-cm^3 gas samples. Typical time intervals between sampling were 20, 40, and 60 min, and 3, 7, and 24 hr, respectively. The concentration of hydrocarbons in the gas phase was determined from the peak heights against appropriate calibration curves. Since hydrogen is evolved during the reaction it proved advantageous to withdraw all gas samples at the same pressure (1 atm). This was achieved by allowing the gases in the reaction tubes to expand into empty 50-ml syringes. After sample withdrawal the gas in the syringe was injected back into the reaction tubes.

Catalytic Isonitrile Reduction with I–$NaBH_4$. Stock solutions of I were prepared by dissolving 0.630 g of crystalline $Na_2Mo_2O_4(Cys)_2 \cdot 5H_2O$ in 20 ml of 0.2 *M* borate buffer of pH 9.6. The solution was purged for 15 min with pure argon and stored in a 25-ml glass vial fitted with rubber septum. Varying amounts of this solution were injected into 25-ml glass vials. The solutions were diluted with 0.2 *M* borate buffer to a total volume of 3.0 ml. Into each vial 0.04 ml of substrate, *e.g.*, CH_3NC, was added by means of a syringe. After this, 0.5 ml of freshly prepared $NaBH_4$ solution in 0.2 *M* borate buffer was injected to initiate the reaction. The gas above the reaction solution was analyzed by the standard glpc technique described above. The formation of primary amines as by-products of the isonitrile reductions was ascertained by analysis of the gas phase and application of the ninhydrin test.

Effect of ATP and of Other Phosphorylating Agents. To establish the effects of ATP, 0.5 ml of freshly prepared 1.2 *M* ATP solution was injected immediately prior to the addition of $NaBH_4$. The reaction products were analyzed by glpc as described above. The isonitrile reduction experiments with other phosphorylating agents, as well as those conducted in D_2O, were carried out in a similar fashion.

pH Dependence of CH_3NC Reduction. To each of seven 25-ml septum-fitted vials was added 1.5 ml of 0.25 *M* cysteine and 1.5 ml of 0.25 *M* Na_2MoO_4 solutions prepared in phosphate buffer (0.2 M) of pH ranging from 5 to 12. All vials were purged with argon for 15 min, and 0.04 ml (0.32 mmol) of CH_3NC was added into each vial *via* a microsyringe. The reaction was initiated by adding 0.5 ml of a fresh 1.33 *M* solution of $NaBH_4$ in the respective buffer, and product yields were determined after 30 min of incubation at 27°. The pH of the reaction mixtures was redetermined immediately after chromatography and was found to correspond to the original values. A similar series of experiments was carried out with $Na_2S_2O_4$ as reducing agent. The results are represented graphically in Figures 2 and 3.

Inhibition Experiments. The inhibiting effect of CO and N_2 in isonitrile reductions was determined relative to argon. Five 25-ml glass vials equipped with rubber septum caps were prepurged with CO, and five others with N_2 and Ar, respectively. To each vial, 3.0 ml of a freshly prepared solution of I in pH 9.6 borate buffer was added, followed by additional purging of the vials with the respective gases for 10 min. Subsequently, the isonitrile substrates were added in each set of vials by means of a syringe, in amounts of 0, 5, 10, 20, or 40 μl, for example, of freshly prepared 10% solutions in H_2O (for CH_3NC) or DMF (for the water-insoluble substituted isonitriles). After 5 min of standing 0.5 ml of freshly prepared 1.33 *M* $NaBH_4$ in pH 9.6 borate buffer was added. After 30 or 60 min of reaction the gaseous phase was assayed for hydrocarbons by glpc after pressure equalization.

Isonitrile Reduction in System Metal Salt–Cys. Solutions of the metal salts (0.1 *M*, in 0.2 *M* borate buffer) were prepared prior to the experiments. Argon-filled 25-ml glass vials equipped with serum caps were filled with 2.5 ml of the metal salt solutions followed by 1.0 ml of a freshly prepared 0.25 *M* solution of cysteine in 0.2 *M* borate buffer. The vials were subsequently purged with argon for 5 min, after which 0.04 ml (0.67 m*M*) of CH_3NC was added by means of a microsyringe. At this point, 0.5 ml of freshly prepared 1.33 *M* $NaBH_4$ solution in pH 9.6 borate buffer was added in the experiments without ATP. In the experiments with ATP, 0.5 ml of a 1.2 *M* ATP solution in 0.2 *M* borate buffer was added immediately before injecting the $NaBH_4$ solution. The results are summarized in Table VI.

Isonitrile Reduction in the System Fe^{2+}–Cys. Stock solutions of 0.1 *M* $FeSO_4$ in water and of Cys hydrochloride (in 0.2 *M* borate buffer, pH 9.6) were prepared and stored under argon. Varying known amounts of Fe^{2+} and Cys were injected into argon-filled, rubber septum capped, 25-ml glass vials, maintaining the total volume of the two solutions at 3.0 ml. The subsequent operations were identical with those described for the study of the system MoO_4^{2-}–Cys. The results are represented graphically in Figure 4. The cocatalyst effect of iron was determined under conditions described above, except that molybdenum was replaced by iron (added as $FeSO_4 \cdot 7H_2O$). The results are shown graphically in Figure 11.

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Crystal and Molecular Structure of Methyl Pheophorbide with Applications to the Chlorophyll Arrangement in Photosynthetic Lamellae[1]

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Abstract: The structure of methyl pheophorbide *a*, $C_{36}H_{38}N_4O_5$, a derivative of chlorophyll, has been determined by single-crystal X-ray diffraction. The crystals are monoclinic, space group $P2_1$, with $a = 8.035$ (4), $b = 28.531$ (20), and $c = 7.320$ (4) Å, $\beta = 110.96$ (6)°, and $Z = 2$. The structure was solved by trial-and-error methods and refined by block-matrix techniques to a conventional R of 0.051 for 1616 independent nonzero reflections measured with a manual diffractometer. Thirty-five of the 38 hydrogen atoms were included in these calculations. The hydrogen atoms bonded to nitrogen are disordered. There are no chlorin–chlorin intermolecular contacts less than 3.5 Å. A model for the arrangement of chlorophyll molecules in photosynthetic lamellae is proposed in which nonparallel chlorophyll molecules are related by a 2_1 screw axis and are linked by water molecules.

Lack of sufficiently large crystals has hindered X-ray diffraction studies of chlorophyll, but structures have been reported for several more or less related compounds including porphin,[2] two modifications of tetraphenylporphin,[3,4] a magnesium tetraphenylporphin hydrate,[5] phthalocyanin,[6] and a magnesium phthalocyaninpyridine hydrate.[7] More similar to chlorophyll are a phyllochlorin ester[8] which has the chlorin moiety of chlorophyll (*i.e.*, a saturated bond in ring IV) but which lacks the fifth isocyclic ring, and vanadyldeoxophylloerythroetioporphyrin[9] which has the isocyclic ring but is unsaturated in ring IV.[10]

We became interested in methyl pheophorbide *a* ($C_{36}H_{38}N_4O_5$, Figure 1) because of its structural similarity to chlorophyll and the availability of crystals. It differs from chlorophyll *a* only by having two hydrogen atoms rather than magnesium in the center and a methyl ester rather than a phytyl ($C_{20}H_{39}$) ester on the side chain off ring IV.[11] This paper reports our results concerning the crystal structure and molecular geometry of this substance, as well as a proposed model for the packing arrangement of chlorophyll in photosynthetic lamellae.

Experimental Section

Methyl pheophorbide *a* (MePPb) was obtained from R. B. Woodward in 1961. The red-violet flakes, too small for X-ray diffraction measurements, were recrystallized from a 1:1 benzene–methanol solution by slow evaporation to dryness in the dark. The resultant opaque crystals resembled soft lumps of coal with dimensions as great as 2 mm. A wedge-shaped fragment which measured 0.2 × 0.1 × 0.05 mm was detached with a razor blade and used for both the determination of cell dimensions and the collection of data.

Weissenberg photographs of this fragment indicated Laue symmetry 2/m. The intrinsic molecular noncentricity and the systematic absences on Weissenberg photographs ($0k0$, $k \neq 2n$) imply that the probable space group is $P2_1$. Both the cell dimensions and intensity data were measured with a General Electric XRD-5 X-ray diffractometer equipped with a copper tube, a manual quarter-circle Eulerian-cradle goniostat, and a 0.003-in. thick nickel filter at the receiving slit. The distances from the crystal to the X-ray source and to the receiving slit are 14.5 and 17.8 cm, respectively. The crystal was aligned with (101) normal to the instrumental φ axis. The χ, φ, and 2θ values of 25 reflections, for each of which the α doublet was resolved ($\lambda = 1.5405$ Å for Cu $K\alpha_1$), were used in a least-squares refinement of the unit cell parameters and crystal orientation. The unit cell dimensions (at room temperature, ~23°) are $a = 8.035 \pm 0.004$, $b = 28.531 \pm 0.020$, and $c = 7.320 \pm 0.004$ Å, and $\beta = 110.96 \pm 0.06$°. A density of 1.25 ± 0.01 g/cm³ was obtained from flotation in aqueous solutions of $ZnBr_2$, KBr, and NaI. The calculated density is 1.285 for a formula weight of 606.7, $Z = 2$, and $V = 1567$ Å³.

Each reflection in half a sphere in reciprocal space corresponding to spacing $\geqq 1.006$ Å ($2\theta \leqq 100°$) was counted for 10 sec with crystal and counter stationary and at a takeoff angle of 4°. Individual backgrounds were measured for reflections seriously affected by streaking from lower orders; for the rest, backgrounds were taken from a plot of background as a function of Bragg angle. Of the 3214 reflections measured, the intensities of 3103 were above background. The rms difference between $I(hkl)$ and $I(\bar{h}\bar{k}\bar{l})$ was 6.2% of $I(hkl)$. The intensities of the Bijvoet pairs were averaged to produce 1662 independent reflections of which 1616 had net intensities greater than zero. The intensities of three standard reflections, measured periodically, varied by less than ±2%. No correction was made for absorption ($\mu = 6.9$ cm^{-1}).

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