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Nickel Enzymes

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Nickel is a relatively abundant element, ca. 8% of the earth's core and 0.01% of the earth's crust, and is readily available to organisms by leaching of the most abundant form, nickel(II) ions (National Research Council, 1975). This availability notwithstanding, no protein containing functionally significant nickel was known until 1975. As of this writing, four nickel-containing enzymes have been detected and isolated, as noted in Table I. Stoichiometric quantities of nickel have been detected in these enzymes by atomic absorption, by EPR[1] analysis on ^{61}Ni- ($I = {}^3/_2$) enriched proteins, and by use of the radioactive ^{63}Ni isotope (β-emitter, 0.067 MeV, $t_{1/2} = 92$ years).

The first enzyme, jack bean urease, was available for 50 years in crystalline form before the catalytically required nickel (two per subunit) was detected (Dixon et al., 1975). The nickel(II) site appears to have nitrogen or oxygen ligands by EXAFS analysis (Alagna et al., 1984), and the two nickels have been postulated to act differently, one to polarize the urea carbonyl group and the other to increase the nucleophilicity of a coordinated water molecule for attack on the same carbonyl of urea (Andrews et al., 1984; Blakely & Zerner, 1984) (Figure 1). Recently, nickel has also been detected in all purified bacterial ureases [for a review, see Hausinger (1987)]. Why nickel(II) is used uniquely in urease rather than the zinc(II) employed for similar catalytic roles in many other enzymes remains to be determined. In contrast to the role of nickel in the other three enzymes of Table I, there is no indication that nickel(II) ureases use redox chemistry during catalysis of the hydrolysis of urea.

Following the seminal observation of Graf and Thauer (1981) that methanogenic bacteria have an obligate requirement for nickel ions for growth, it was rapidly established that nickel is stoichiometrically present in three heterooligomeric enzymes, hydrogenases [see Hausinger (1987)], methyl coenzyme M reductase (Gunsalus & Wolfe, 1980; Ellefson et al., 1982), and an acetyl-CoA-cleaving CO dehydrogenase (Krzycki & Zeikus, 1984). In rapid succession hydrogenases from many other bacteria, such as *Chromatium* (Albracht et al., 1982), *Alcaligenes* (Fredrich et al., 1982), *Desulfovibrio* (Kruger et al., 1982; LeGall et al., 1982), *Azotobacter* (Seefeldt & Arp, 1986), *Nocardia* (Schneider et al., 1984), and *Escherichia coli* (Ballantine & Boxer, 1985), were reanalyzed and found to harbor stoichiometric amounts of nickel. The nickel-containing CO dehydrogenase is also found in acetogenic bacteria and has been best characterized from *Clostridium thermoaceticum* (Ragsdale et al., 1983b,c).

Despite the lack of compelling precedent for the utilization of nickel for the hydrolysis of urea, we feel it is no accident that nickel has been incorporated into the latter three enzymes of Table I, which function physiologically as a biological *hydrogenation* catalyst, *desulfurization* catalyst, and *carbonylation* catalyst, respectively. Industrial nickel catalysts effect each of these transformations, and no other *redox-active* metal commonly available to organisms from leaching of the earth's crust can substitute for nickel in these transformations. Nickel appears to possess a unique combination of redox and coordination properties leading to its selection for the biological analogues of the industrial processes mentioned.

Given the quite recent purification of the three redox enzymes and detection of stoichiometrically bound nickel, it is not yet known exactly how nickel may be used for (a) hydrogen gas oxidation, (b) methyl thioether reductive desulfurization to methane, and (c) carbonylation of a methyl group to synthesize acetyl-CoA. Such studies will doubtless reveal novel bioinorganic and biorganometallic enzyme chemistry. To understand how nickel in one enzyme microenvironment may be involved in the splitting of H_2, in another be involved in C–S thioether net protonolytic cleavage, and in a third use ligated CO to insert into a methyl ligand will require a detailed knowledge of the enzymes' active-site structures. This will involve identification of ligands to nickel in resting enzyme

[1] Abbreviations: EPR, electron paramagnetic resonance; EXAFS, extended X-ray absorption fine structure spectroscopy; CoA, coenzyme A; FAD, flavin adenine dinucleotide; MV, methylviologen; HPLC, high-pressure liquid chromatography; CoM, coenzyme M; THF, tetrahydrofolic acid; MCD, magnetic circular dichroism; ENDOR, electron nuclear double resonance.

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Table I: Nickel Enzymes

enzyme	source	cofactor content	nickel site	postulated role for nickel
(1) urease	plants, bacteria	2Ni/subunit	oxygen or nitrogen ligands from protein	acid catalysis and H_2O activation
(2) hydrogenases	many bacteria	nickel, iron–sulfur clusters (FAD occasionally)	nickel tetrathiolate in distorted tetragonal array	site for H_2 binding and fragmentation
(3) methyl coenzyme M reductase	methanogenic bacteria	nickel tetrahydrocorphin (F_{430})	nickel tetrapyrrole coenzyme F_{430}	desulfurization catalyst
(4) CO dehydrogenase (acetyl-CoA decarbonylating)	acetogenic and methanogenic bacteria	nickel, zinc; iron–sulfur clusters	mixed nickel–iron cluster proposed	carbonylation catalyst for C–C bond formation and cleavage

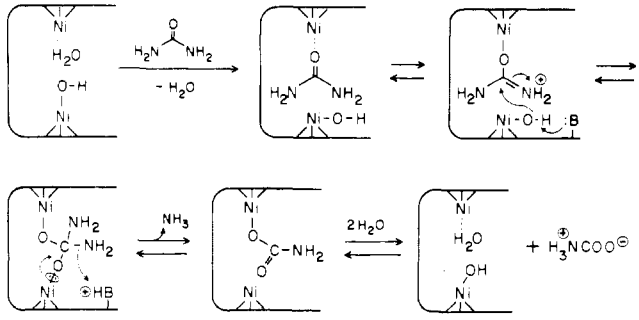

FIGURE 1: Proposed mechanism for utilization of two nickel atoms in urease catalysis, redrawn from Blakeley and Zerner (1984).

and in enzyme–substrate, enzyme–intermediate, and enzyme–product complexes. The distance and geometry of each ligand to nickel, the oxidation states traversed by nickel, and the identity of the ligands must be determined. While Ni^{II} (d^8) is the common oxidation state in aqueous solutions, Ni^0–Ni^{IV} oxidation states are known, with different preferred geometries and different ligation preferences. For example, Ni^I tends to be four-coordinate and tetrahedral, while Ni^{II} may be four- or six-coordinate and tetrahedral, square planar, or octahedral. The d^7 Ni^{III} ion prefers five- and six-coordination and prefers to be trigonal bipyramidal or distorted octahedral. Thus a net two-electron redox change at an enzyme Ni site, e.g., from Ni^{III} to Ni^I, might well involve substantial reorganization at the site, and restraints imposed by the protein–ligand system would surely control the chemistry enabled.

The nickel-containing hydrogenases, methyl coenzyme M reductase, and CO dehydrogenases are complex multimeric enzymes, inactivated in air. The presence of other redox cofactors (Fe, FAD) and other metals (Zn) and multiple subunits (such that localization of nickel to a given subunit is not yet well established) complicates analysis and makes X-ray structures unlikely in the near future.

No data yet exist on *reversible* removal of nickel from these enzymes or its replacement or substitution by other metals or functional reconstitution of any activity by exogenous nickel. Nor have reports appeared on nickel-specific chelation and removal to a denickel enzyme form. Initial efforts on the hydrogenases and CO dehydrogenase to removal nickel induced irreversible loss of all cofactors from the protein. We shall analyze each nickel enzyme in turn, considering what is currently known about the nickel site, what mechanistic roles may be ascribable to nickel in catalysis, and experiments that bear on such proposals. We discuss the known enzyme facts, precedents for nickel-based catalysis from the inorganic and organometallic literature, and distinguish them from mechanistic hypotheses for the enzymes.

Nickel-Containing Hydrogenases

While all known hydrogenases contain Fe–S clusters (Adams et al., 1981), many, but importantly *not* all (Adams & Mortenson, 1984; Huynh et al., 1984), have now been found to contain nickel as well, and nickel may be a common constituent of hydrogenases that function physiologically to oxidize rather than evolve H_2. Because hydrogenases without nickel exist, one must be cautious about any proposal that assigns an important or essential role to nickel in catalysis until rigorously proven, and none have been to date. The best characterized hydrogenases are either from sulfate-reducing bacteria of the *Desulfovibrio* genus (Teixeira et al., 1985) or from the methanogenic bacterium *Methanobacterium thermoautotrophicum* (Bastian et al., 1987a,b), which uses H_2 as the sole oxidizable energy source. The *Desulfovibrio vulgaris* enzyme is an α, β dimer (46K, 14K), and the gene sequence of the large subunit has been determined (Voordouw & Brenner, 1985).

Methanobacteria (and other bacteria) have multiple hydrogenases, and we have purified two to homogeneity from *M. thermoautotrophicum* (Jacobson et al., 1982; Fox et al., 1987; Jordan, 1985). One uses the 8-hydroxy-5-deazaflavin coenzyme F_{420} (Eirich et al., 1978; Walsh, 1986) as cosubstrate while the other is assayed by reduction of the artificial dye methylviologen. The former contains three Fe_4S_4 clusters, one Ni, and one FAD per α (47K), β (31K), γ (26K) unit (Fox et al., 1987) but appears to be mostly an α8–β8–γ8 complex, with a characteristic morphology by electron microscopic analysis (Wackett et al., 1987c). The latter hydrogenase appears to have at least four subunits, α (57K), β (45K), γ (42K), and δ (33K) (A. Kolodjiez, C. Walsh, and W. Orme-Johnson, unpublished data in a collaborative effort with the group of J. Reeve at Ohio State). An F_{420} nonreactive MV-reducing hydrogenase has also been purified from *Methanobacterium formicicum*, a two subunit (48K, 38K) enzyme (Jin et al., 1983; Adams et al., 1986). EPR analysis indicates a similar but not identical Ni site (Tan et al., 1984; Adams et al., 1986) and a 3Fe center (Jordan, 1985; Adams et al., 1986). Cloning and sequencing of these hydrogenase genes are in progress.

The *Desulfovibrio* and methanobacterial hydrogenases, among others (Hausinger, 1987), show nickel EPR signals on isolation, documented by hyperfine coupling in ^{61}Ni- ($I = {}^3/_2$) containing preparations and assigned to a nickel(III) oxidation state in an air-oxidized, inactive enzyme form (Moura et al., 1982; Kojima et al., 1983). Anaerobic reductive activation of enzyme with H_2 converts the nickel to an EPR-silent form, probably by nickel reduction. Extended X-ray absorption fine structure spectroscopy (EXAFS) of these hydrogenases indicates a sulfur-rich nickel site and a distorted octahedral symmetry with four sulfurs as likely equatorial ligands to nickel [at 2.02–2.3-Å distances (Lindahl et al., 1984; Scott et al., 1984)] and an additional axial coordination site or two. Whether this tetrathiolate nickel site is the site for H_2 binding and oxidative fragmentation is under active study, and mechanistic proposals have been made for both heterolytic and homolytic H_2 bond breakage and for oxidation states of nickel from Ni^{IV} to Ni^0 [see Teixeira et al. (1985) and Bastian et

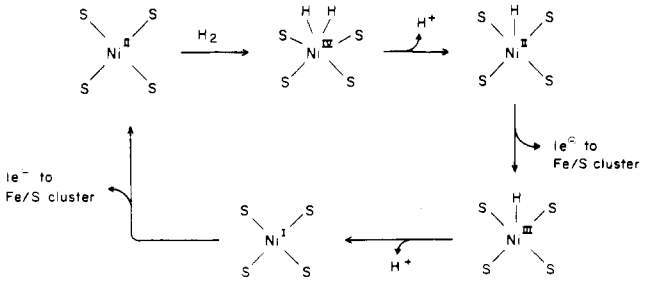

FIGURE 2: Possible redox cycle for the nickel site in methanogenic bacterial hydrogenases. The initial binding of H_2 and subsequent proposed proton dissociations could involve formal oxidation states of nickel from IV to I.

al. (1987a,b) for review]; one such route is shown in Figure 2. If nickel turns out to be the catalytic site for H_2 oxidation, the Fe–S clusters will probably serve as one-electron oxidants of the bound nickel.

Nickel-Dependent Methyl-CoM Reductase

The last step in the generation of CH_4 by methanogenic bacteria is the exergonic cleavage of the thioether dimethyl 2,2′-dithiobis(ethanesulfonate) (methyl-CoM) to methane and CoMSH, catalyzed by an enzyme methyl-CoM reductase Ellefson & Wolfe, 1981). Studies of this reductive desulfurization step have been hampered by very low in vitro rates and the requirement for several additional oxygen-sensitive components to achieve detectable in vitro methanogenesis, but persistent efforts by Wolfe and colleagues have defined the need for three additional proteins [one apparently the F_{420}-reducing hydrogenase (vide supra)], for Mg substoichiometric amounts of ATP (Nagle & Wolfe, 1983), and for a factor B [identified as (7-mercaptoheptanoyl)threonine phosphate (Noll et al., 1986, 1987)]. Most recently, Thauer's group has achieved reconstitution, albeit at 0.3% in vivo rates of methanogenesis, with pure reductase, factor B, dithiothreitol, and vitamin B_{12} (Ankel-Fuchs & Thauer, 1986), and this may be a tractable system in which to study the mechanism [but see Whitman and Wolfe (1987)].

Methyl-CoM reductase constitutes ca. 10% of the soluble cell protein in *M. thermoautotrophicum* and can be purified to homogeneity in 100-mg quantities (Hausinger et al., 1984). With this preparation and all other reported preparations of this enzyme it is found that in vitro less than 1% of the activity, expected from the methanogenic capacity of cells and the content of this protein, can be demonstrated. One is forced to conclude that an enzyme component has been lost or that most of the molecules are inactivated during purifications reported to date. For this reason studies of the kinetic mechanism and catalytic chemistry may be worthwhile, but biophysical studies on the nearly inactive preparation may not be revealing of relevant active-site detail. The enzyme is an α_2 (68K)–β_2 (47K)–γ_2 (38K) complex of aggregate molecular weight 300000, and the three encoding genes are in an operon organization (Konheiser et al., 1984; Cram et al., 1987). The enzyme has a yellow color, stemming from two tightly associated molecules of a nickel tetrapyrrole macrocyclic coenzyme F_{430} associated with the α subunit (Hartzell & Wolfe, 1986). Enzyme-bound CoF_{430} has a λ_{max} of 418 nm but when released by mild denaturation has a λ_{max} at 430 nm (ϵ = 23 300 M^{-1} cm^{-1}). The reductase as isolated typically also contains one molecule of CoM, possibly bound as a mixed cysteine disulfide (Keltjens et al., 1982; Ellefson & Wolfe, 1981), and varying amounts of factor B (Noll & Wolfe, 1986).

Coenzyme F_{430} is the first nickel tetrapyrrole coenzyme discovered, and its structure and properties have been determined in a monumental joint study by the Eschenmoser and Thauer groups (Pfaltz et al., 1982, 1985), which is the benchmark for any biological interpretation. Pfaltz et al. (1982) have termed the F_{430} structure a tetrahydro*corphin* to emphasize the hybrid relationship to corrin and porphyrin macrocyclic structures and to suggest the nickel-containing corphin is a "missing link" between iron-containing porphyrin and cobalt-containing corrin systems. Sirohydrochlorin, a B_{12} precursor, is converted to F_{430} as well in *M. thermoautotrophicum* cell-free extracts (Mucha et al., 1985). As shown in Figure 3, the pyrrolic A ring has a fused δ-lactam and the pyrrolic C ring has a fused cyclohexanone, functional modifications that help distort the macrocycle away from planarity to a ruffled or puckered structure. This nonplanarity of the tetrahydrocorphin macrocycle has profound consequences on nickel reactivity (Pfaltz et al., 1985). Notably, in contrast to nickel in planar porphyrins, which are square planar four-coordinate with no tendency to axial reactivity, the nickel corphinoids are axially reactive (Fassler et al., 1984). An X-ray structure of a thiocyanate complex of an F_{430} analogue shows six-coordinate nickel and Ni–N distances of 2.08 Å (Kratky et al., 1984; Waditschatka et al., 1985), considerably longer than the 1.91-Å distance from nickel to equatorial nitrogen ligands in four-coordinate nickel porphyrins. Nickel EXAFS studies on F_{430} in solution and bound to methyl-CoM reductase also show Ni^{II}–N distances of 2.06–2.09 Å (Diakum et al., 1985; Eidsness et al., 1986; Orme-Johnson, Bastian, Niederhoffer, Hausinger, and Walsh, in preparation) and likely six-coordinate behavior. In passing, it is noted that coenzyme F_{430} is labile to isomerization to yield 12-epi or 12,13-epi isomers which still absorb at 430 nm, but these artifactual isomers are separable by HPLC (Pfaltz et al., 1985).

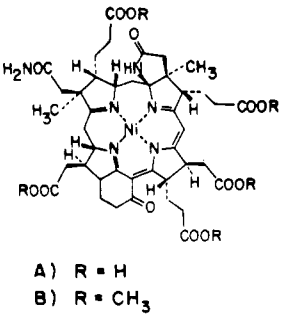

FIGURE 3: Structure of the tetrahydrocorphinoid nickel-containing coenzyme F_{430}. The pentamethyl ester was the initial derivative characterized but the pentaacid is the active coenzyme form bound to methyl-CoM reductase.

A major mechanistic issue in methyl-CoM reductase catalysis is how the enzyme modulates the reactivity of the Ni F_{430} coenzyme. As indicated above, this cannot yet be addressed with complete confidence by using purified reductase of low specific activity. Recent studies using ^{61}Ni-labeled cells and purified enzyme detect an EPR-active form of F_{430}, presumably a Ni^{I} form [Albracht et al., 1986; also see Jaun and Pfaltz (1986)]. The nickel paramagnetic concentration is low compared to the Ni content, but shows some correlation with in vitro methanogenic activity, so one-electron reduction of Ni^{II} to Ni^{I} in bound F_{430} may be catalytically consequential. In this connection Raney nickel catalyzed desulfurizations are thought to proceed by radical pathways as an explanation of observed coupling products, disproportionations, fragmentations, and racemizations [Bonner & Grimm, 1966; see Wackett et al. (1987a,b)].

As yet, no intermediates have been detected in methyl reductase catalysis, but there are precedents in nickel chemistry for nickel thioether complexes, nickel hydride complexes, and

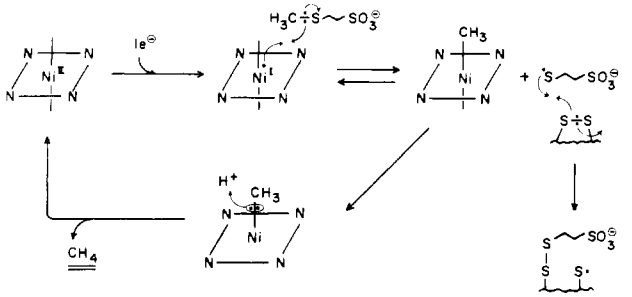

FIGURE 4: Possible mechanism for methyl coenzyme M reductase involving one-electron reduction to a Ni(I) F_{430} species, formation of a methyl–nickel intermediate, and protonolytic decomposition.

organonickel species [see Wackett et al. (1987a)] and one or more of these may figure in methane formation. To exemplify just the last possibility, there is the precedent of the organocobalt species, methyl coenzyme B_{12}, in the methyl transfer from N^5-methyl-THF to homocysteine by methionine synthase (Taylor & Weissbach, 1969). The initial Co^{III} oxidation state is reduced (by one-electron steps?) to Co^{I}, which, as a supernucleophile, reacts with N^5-CH_3-THF to yield the methyl–cobalt species. Methyl B_{12} is stable enough to be synthesized and was shown to be a competent intermediate. Analogously, Ni^{II} F_{430} may be reduced in turnover to the EPR-detectable Ni^{I}, which could then react with a methyl-CoM molecule at the active site of methyl reductase by a homolytic C–S fragmentation to produce methyl F_{430} and a CoM thiyl radical. If the CoMS$^{\bullet}$ reduced an enzyme disulfide, the CoMS–Senz mixed disulfide (found in isolated methyl-CoM reductase) would be generated as the initial coproduct (Figure 4). In contrast to stable organocobalt species in tetrapyrroles, corresponding organonickel tetrapyrroles are labile to protonolysis and, for example, methyl–nickel species yield methane (Callot et al., 1975; D'Aniello & Barefield, 1976; Wilke & Herrman, 1966). To test this mechanistic proposal and others for the function of this biological version of Raney nickel will first require a preparation of population of *fully active* enzyme molecules, followed by analysis of nickel oxidation states and axial ligands in turnover and detection of any transient covalent nickel compounds. Analogues of methyl-CoM that might give mechanistically diagnostic products (e.g., racemization at the alkyl group) would be useful, depending on the strictures of enzyme specificity. Thus while ethyl-CoM yields ethane (Gunsalus et al., 1978), propyl- and cyclopropylmethyl-CoM are not substrates (Wackett et al., 1987a). Difluoromethyl-CoM is processed exclusively to difluoromethane, arguing against difluorocarbene-type intermediates since those lose F^- (Wackett et al., 1987a).

Nickel-Containing Carbon Monoxide Dehydrogenase

Enzymes interconverting CO and CO_2 in the presence of redox dyes such as methylviologen have been purified from methanogenic bacteria and acetogenic bacteria, and of these the best characterized is the CO dehydrogenase from *Clostridium thermoaceticum*, an α_3, β_3 heterotrimer (78K, 71K) of aggregate molecular weight ca. 400 000, comprising six nickel ions, three zinc ions, and 32–40 iron and sulfide sulfur atoms (Ragsdale et al., 1983a–c). The CO dehydrogenases appear to be crucial catalysts in acetyl-CoA synthesis from two C_1 fragments in both methanogens and acetogens. The enzyme is also central to the retrograde process, the fragmentation of acetate, in acetate-grown cells (Diekert et al., 1985; Zeikus et al., 1985; Ljungdahl, 1986; Wood et al., 1986), ultimately to CH_4 and CO_2. For example, in *C. thermoaceticum* CO_2 is reduced to CO, possibly at the nickel site of CO dehydrogenase, while the CH_3 group from N^5-CH_3-THF is transferred to a corrinoid protein to yield a methyl B_{12} protein that is the actual donor of the CH_3 fragment. The C–C bond forming step occurs in the active site of the CO dehydrogenase and is a *net carbonylation*, the first biological example of such a C–C formation, carbonylations being of course well precedented in organometallic chemistry (Collman & Hegedus, 1980).

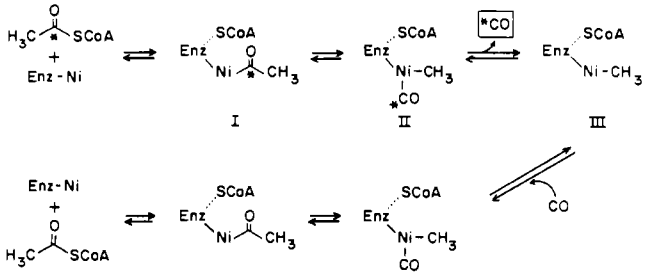

FIGURE 5: Mechanistic proposal to account for the reversible decarbonylation of acetyl-CoA by the nickel enzyme CO dehydrogenase. Formation of an acetyl–nickel species and its conversion to a methyl–nickel species with an adjacent, dissociable CO ligand to nickel is precedented in nonenzymic nickel chemistry.

The crucial evidence for *C. thermoaceticum* CO dehydrogenase as a biocarbonylation reagent stems from two recent isotope-exchange studies by Ragsdale and Wood (1985) and Pezacka and Wood (1986). One exchange demonstrated to be catalyzed by pure enzyme was a [^{3}H]CoASH–acetyl-CoA exchange, suggesting reversible formation of an *acetyl-enzyme* intermediate. The more remarkable exchange was enzymic "washout" of 1-[^{14}C]carbonyl-labeled acetyl-CoA in the presence of excess CO, suggesting the enzyme can reversibly cleave the acetyl-CoA into a *methyl fragment*, a ^{14}CO fragment, and a CoAS fragment, exchange the ^{14}CO with free CO in solution, and re-form the acetyl-CoA. This remarkable decarbonylation/carbonylation sequence is hitherto unprecedented in enzymology but on the organometallic precedents strongly suggests organometal intermediacy (e.g., at nickel or at iron) (Figure 5). The nickel possibility is exemplified where acetyl-CoA could yield an acetyl–nickel, then a methyl–nickel with adjacent CO ligand, and then a methyl–nickel intermediate during CO exchange. This proposed enzyme chemistry is closely precedented by organonickel chemistry described by Kohara et al. (1979), who exposed a methyl–nickel compound to 1 equiv of CO and crystallized an acetyl–nickel carbonylation product, which then reacted with *p*-cyanophenol to undergo acetyl transfer to the activated ester *p*-cyanophenyl acetate as a model for acetyl-CoA formation. We have recently tested this possibility using *R*- and *S*- (H,D,T) chiral methylacetyl-CoA samples. Carbonylation of organoiron and organopalladium compounds is known to proceed with retention of configuration at the migrating alkyl group [see Collman and Hegedus (1980)]. Although no stereochemical outcomes are yet known for *nickel* carbonylations, retention is also expected and is the observed result when (*R*)- and (*S*)-acetyl-1-[^{14}C]acetyl-CoA samples are reversibly decarbonylated by *C. thermoaceticum* CO dehydrogenase and the C_2 stereochemistry of reisolated acetyl-CoA is analyzed (Raybuck et al., 1987). Subsequent studies must focus on direct detection of the proposed acetyl–metal (nickel or iron) and methyl–metal intermediates, experimentally exacerbated by the extreme sensitivity to enzyme inactivation by oxygen and the lability of the carbonylation exchange activity, which is inactivated *much* more rapidly than the CO–CO_2 dehydrogenation activity (Raybuck et al., 1987).

The nickel site is as yet poorly characterized in the *C. thermoaceticum* CO dehydrogenase and even less so in the *Methanosarcina barkeri* enzyme. Nickel EXAFS reveals a sulfur-rich nickel site (N. Bastian, C. Walsh, and W. Orme-Johnson, unpublished data), and the finding of ^{57}Fe nuclear hyperfine coupling on the Ni-centered EPR signal of the CO complex has led to the tentative proposal of a mixed nickel–iron cluster (Ragsdale et al., 1985), an exciting prospect that will require careful substantiation. Static studies with ^{61}Ni enzyme and ^{13}CO show line broadening on the nickel EPR signals, consistent with an anticipated Ni–CO complex, but kinetic competence of this intermediate remains to be established, and nothing has yet been reported on what enzyme group (nickel and/or an active-site residue) is reversibly acetylated, though this is an obvious line of inquiry.

Conclusions

Nickel as a stoichiometric component in enzymes has only been realized within the past 12 years, and it may be that other examples will be detected. In any case nickel has moved rapidly to center stage in redox biochemistry. It is not clear yet why Ni^{II} is used in place of Zn^{II} in urease, but we argue there is no other single biologically available metal that could replace nickel in the other three enzymes where nickel redox chemistry is almost surely in play in biohydrogenation catalysis, reductive biodesulfurization catalysis, and biocarbonylation. Because of the complex cofactor content and heterooligomeric structure of these enzymes, knowledge of nickel function in catalysis is still primitive. The delineation of the active-site nickel structures will be complex and will doubtless require sophisticated spectroscopic approaches (e.g., EPR, EXAFS, MCD, ENDOR) coupled to gene cloning, subunit expression, and nickel-site reconstitution. The answers to such questions are important for they should reveal how the three enzyme microenvironments direct nickel chemistry to three distinctive reaction manifolds. Lastly, we note that methanogenic bacteria (which have all three nickel redox catalysts) are members of the archaebacterial kingdom (Woese & Olsen, 1986) and represent descendents of primordial organisms who we presume learned 3 billion years ago how to use nickel chemistry to grow chemolithioautotrophically on H_2 and CO_2. Explication of the nickel sites of hydrogenases, methyl-CoM reductase, and CO dehydrogenase may provide insights leading to the perfection of homogeneous and heterogeneous manmade nickel catalysts, which to date have been less than 100 years in development.

Registry No. Ni, 7440-02-0; urease, 9002-13-5; hydrogenase, 9027-05-8; methyl coenzyme M reductase, 53060-41-6; CO dehydrogenase, 64972-88-9; coenzyme F_{430}, 73145-13-8.

References

Adams, M. W., & Mortenson, L. E. (1984) *J. Biol. Chem. 259*, 7045–7055.

Adams, M. W., Mortenson, L. E., & Chen, J. S. (1981) *Biochim. Biophys. Acta 594*, 105–176.

Adams, M. W., Jin, S. L., Chen, J. S., & Mortenson, L. E. (1986) *Biochim. Biophys. Acta 869*, 37–47.

Alagna, L., Hasrain, S. S., Piggott, B., & Williams, D. J. (1984) *Biochem. J. 220*, 591–595.

Albracht, S. P. J., Graf, E. G., & Thauer, R. K. (1982) *FEBS Lett. 140*, 311–313.

Albracht, S. P. J., Ankel-Fuchs, D., Van der Zwaan, J. W., Fontija, R. D., & Thauer, R. K. (1986) *Biochim. Biophys. Acta 832*, 89–97.

Andrews, R. K., Blakeley, R. L., & Zerner, B. (1984) *Adv. Inorg. Biochem. 6*, 245–283.

Ankel-Fuchs, D., & Thauer, R. (1986) *Eur. J. Biochem. 155*, 156–160.

Ballantine, S. P., & Boxer, D. H. (1985) *J. Bacteriol. 163*, 454–459.

Bastian, N. R., Livingston, D. J., Jordan, L M., Fox, J., Wackett, L. P., Orme-Johnson, W. H., & Walsh, C. T. (1987a) in *Bioinorganic Chemistry of Nickel* (Lancaster, J., Ed.) (in press).

Bastian, N. R., Wink, D. A., Wackett, L. P., Livingston, D. J., Jordan, L. M., Fox, J., Orme-Johnson, W. H., & Walsh, C. T. (1987b) in *Bioinorganic Chemistry of Nickel* (Lancaster, J., Ed.) (in press).

Blakeley, R., & Zerner, B. (1984) *J. Mol. Catal. 23*, 263–292.

Bonner, W. A., & Grimm, R. A. (1966) in *The Chemistry of Organic Sulfur Compounds* (Kharusch & Meyers, Eds.) pp 410–413, Pergamon, New York.

Callot, H., Tschamber, T., Chevrier, B., & Weiss, R. (1975) *Angew. Chem., Int. Ed. Engl. 14*, 567–568.

Collmann, J., & Hegedus, L. (1980) in *Principles and Applications of Organotransmission Metal Chemistry*, University Science Books, Mill Valley, CA.

Cram, D. S., Sherf, B. A., Libby, R. T., Mattaliano, R. J., Ramachandran, K. L., & Reeve, J. N. (1987) *Proc. Natl. Acad. Sci. U.S.A.* (in press).

D'Aniello, J. J., & Barefield, E. K. (1976) *J. Am. Chem. Soc. 98*, 1610–1611.

Diakum, G. P., Piggott, B., Timton, H. J., Ankel-Fuchs, D., & Thauer, R. K. (1985) *Biochem. J. 232*, 281–284.

Diekert, G., Fuchs, G., & Thauer, R. (1985) in *Microbial Gas Metabolism*, pp 115–130, Academic, New York.

Dixon, N., Gazzola, C., Blakeley, R. L., & Zerner, B. (1975) *J. Am. Chem. Soc. 97*, 4131–4133.

Eidsness, M. K., Sullivan, R. J., Schwartz, J. R., Hartzell, P. L., Wolfe, R. S., Flank, A. M., Cramer, S. P., & Scott, R. A. (1986) *J. Am. Chem. Soc. 108*, 3120–3121.

Eirich, L. D., Vogels, G. D., & Wolfe, R. S. (1978) *Biochemistry 17*, 4583–4588.

Ellefson, W. L., & Wolfe, R. L. (1981) *J. Biol. Chem. 256*, 4259–4262.

Ellefson, W., Whitman, W. B., & Wolfe, R. S. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3707–3710.

Fassler, A., Pfaltz, A., Krautler, B., & Eschenmoser, A. (1984) *J. Chem. Soc., Chem. Commun.*, 1365–1367.

Fox, J., Livingston, D., Orme-Johnson, W., & Walsh, C. (1987) *Biochemistry* (in press).

Friedrich, C. G., Schneider, K., & Friedrich, B. (1982) *J. Bacteriol. 152*, 42–48.

Graf, E. G., & Thauer, R. K. (1981) *FEBS Lett. 136*, 165–169.

Gunsalus, R. P., & Wolfe, R. S. (1980) *J. Biol. Chem. 255*, 1891–1895.

Gunsalus, R. P., Romesser, J. A., & Wolfe, R. S. (1978) *Biochemistry 17*, 2374–2377.

Hartzell, P. L., & Wolfe, R. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6726–6730.

Hausinger, R. (1987) *Microbiol. Rev.* (in press).

Hausinger, R. P., Orme-Johnson, W. H., & Walsh, C. T. (1984) *Biochemistry 23*, 801–804.

Huynh, B., Czechowski, M. H., Kruger, H. J., DerVartanian, D. V., Peck, H. D., & LeGall, J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 3728–3732.

Jacobson, F., Daniels, L., Fox, J. A., Walsh, C. T., & Orme-Johnson, W. H. (1982) *J. Biol. Chem. 257*, 3385–3388.

Jaun, B., & Pfaltz, A. (1986) *J. Chem. Soc., Chem. Commun.*, 3127–1329.

Jin, S. L., Blanchard, D. K., & Chem, J. S. (1983) *Biochim. Biophys. Acta 748*, 8–20.

Jordan, L. (1985) Ph.D. Dissertation, Massachusetts Institute of Technology.

Keltjens, J. T., Whitman, W. B., Caerteling, C. G., van Kooten, A. M., Wolfe, R. S., & Vogels, G. D. (1982) *Biochem. Biophys. Res. Commun. 108*, 495–503.

Kohara, T., Kamiya, S., Yamamoto, T., & Yamamoto, A. (1979) *Chem. Lett.*, 1513.

Kojima, N., Fox, J. A., Hausinger, R. P., Daniels, L., Orme-Johnson, W. H., & Walsh, C. T. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 378–382.

Konheiser, U., Pasti, G., Bollschweiler, C., & Klein, A. (1984) *Mol. Gen. Genet. 198*, 146–152.

Kratky, C., Fassler, A., Pfaltz, A., Krautler, B., Jaun, B., & Eschenmoser, A. (1984) *J. Chem. Soc., Chem. Commun.*, 1368–1371.

Kruger, H. J., Huynh, B. H., Ljungdahl, R., Xavier, A. V., DerVartanian, D. V., Moura, I., Peck, H. D., Teixeira, M., Moura, J. J., & LeGall, J. (1982) *J. Biol. Chem. 257*, 14620–14623.

Krzycki, J. A., & Zeikus, J. G. (1984) *J. Bacteriol. 158*, 231–237.

LeGall, J., Ljungdahl, P., Moura, I., Peck, H. D., Xavier, A. V., Moura, J. G., Teixeira, M., Huynh, B., & DerVertanian, D. V. (1982) *Biochem. Biophys. Res. Commun. 106*, 610–616.

Lindahl, P. A., Kojima, N., Hausinger, R. P., Fox, J. A., Teo, B. C., Walsh, C. T., & Orme-Johnson, W. H. (1984) *J. Am. Chem. Soc. 106*, 3062–3065.

Ljungdahl, L. G. (1986) *Annu. Rev. Microbiol. 40*, 415–450.

Moura, J. J., Moura, I., Huynh, B. H., Kruger, H. J., Teixeira, M., DuVarney, R. C., DerVartanian, D. V., Xavier, A. V., Peck, H. D., & LeGall, J. (1982) *Biochem. Biophys. Res. Commun. 108*, 1388–1393.

Mucha, H., Keller, E., Weber, H., Lingens, F., & Trosch, W. (1985) *FEBS Lett. 190*, 169–173.

Nagle, D. P., & Wolfe, R. S. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2151–2155.

National Research Council (1975) *Nickel*, National Academy of Sciences, Washington, DC.

Noll, K., & Wolfe, R. S. (1986) *Biochem. Biophys. Res. Commun. 139*, 889–895.

Noll, K., Rinehart, K. L., Tanner, R. S., & Wolfe, R. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 4238–4242.

Noll, K., Donnelly, M., & Wolfe, R. (1987) *J. Biol. Chem. 262*, 513–515.

Pezacka, E., & Wood, H. G. (1986) *J. Biol. Chem. 261*, 1609–1615.

Pfaltz, A., Jaun, B., Fassler, A., Eschenmoser, A., Jaenchen, R., Gilles, H. H., Diekert, G., & Thauer, R. (1982) *Helv. Chim. Acta 65*, 828–865.

Pfaltz, A., Livingston, D. A., Jaun, B., Diekert, G., Thauver, R., & Eschenmoser, A. (1985) *Helv. Chim. Acta 68*, 1338–1358.

Ragsdale, S. W., & Wood, H. G. (1985) *J. Biol. Chem. 260*, 3970–3977.

Ragsdale, S. W., Clark, J. E., Ljungdahl, L. G., Lundie, L. L., & Drake, H. L. (1983a) *J. Biol. Chem. 258*, 2364–2369.

Ragsdale, S. W., Ljungdahl, L. G., & DerVartanian, D. V. (1983b) *Biochem. Biophys. Res. Commun. 115*, 658–665.

Ragsdale, S. W., Ljungdahl, L. G., & DerVartanian, D. V. (1983c) *J. Bacteriol. 155*, 1224–1237.

Ragsdale, S. W., Wood, H. G., & Antholine, W. E. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 6811–6814.

Raybuck, S., Bastian, N., Zadowsky, L., Kobayashi, K., Floss, H. G., Orme-Johnson, W. H., & Walsh, C. (1987) *J. Am. Chem. Soc.* (submitted for publication).

Schneider, K., Schlegel, H. G., & Jochim, K. (1984) *Eur. J. Biochem. 142*, 75–84.

Scott, R. A., Wallin, S. A., Czechowski, M., DerVartanian, D. V., LeGall, J., Peck, H., & Moura, I. (1984) *J. Am. Chem. Soc. 106*, 6864–6865.

Seefeldt, L. C., & Arp, D. J. (1986) *Biochimie 68*, 25–34.

Tan, S. L., Fox, J. A., Kojima, N., Walsh, C. T., & Orme-Johnson, W. H. (1984) *J. Am. Chem. Soc. 106*, 3064–3066.

Taylor, R. T., & Weissbach, H. (1969) *Arch. Biochem. Biophys. 129*, 728–744.

Teixeira, M., Moura, I., Xavier, A. V., Huynh, B. H., DerVartanian, D. V., Peck, H. D., LeGall, J., & Moura, J. G. (1985) *J. Biol. Chem. 260*, 8942–8950.

Voordouw, G., & Brenner, S. (1985) *Eur. J. Biochem. 148*, 515–520.

Wackett, L., Honek, J., Begley, T., Orme-Johnson, W. H., & Walsh, C. (1987a) *Biochemistry* (in press).

Wackett, L., Honek, J., Begley, T., Shames, S., Niederhoffer, E., Hausinger, R. P., Orme-Johnson, W. H., & Walsh, C. T. (1987b) in *Bioinorganic Chemistry of Nickel* (Lancaster, J., Ed.) (in press).

Wackett, L., King, J. A., Hartwig, E., Orme-Johnson, W. H., & Walsh, C. (1987c) *J. Bacteriol. 169*, 718–727.

Walsh, C. (1986) *Acc. Chem. Res. 19*, 216–221.

Waditschatka, R., Kratky, C., Jaun, B., Heinzer, J., & Eschenmoser, A. (1985) *J. Chem. Soc., Chem. Commun. 22*, 1604–1607.

Whitman, W. B., & Wolfe, R. S. (1987) *J. Bacteriol. 169*, 87–92.

Wilke, G., & Herrman, G. (1966) *Angew. Chem., Int. Ed. Engl. 5*, 580–581.

Woese, C. R., & Olsen, G. J. (1986) *Syst. Appl. Microbiol. 7*, 161–177.

Wood, H. G., Ragsdale, S. W., & Pezacka, E. (1986) *Trends Biochem. Sci. (Pers. Ed.) 11*, 14–18.

Zeikus, J. G., Kerby, R., & Krzycki, J. A. (1985) *Science (Washington, D.C.) 227*, 1167–1173.