This article was downloaded by:
On: *15 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Comments on Inorganic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455155

F430-Dependent Biocatalysis in Methanogenic Archaebacteria

Hoshik Won[a]; Karl D. Olson[b]; Michael F. Summers[c]; Ralph S. Wolfe[b]
[a] Department of Chemistry, Emory University, Atlanta, Georgia [b] Department of Microbiology, University of Illinois, Urbana, Illinois [c] Department of Chemistry and Biochemistry, University of Maryland, Baltimore, Maryland

To cite this Article Won, Hoshik , Olson, Karl D. , Summers, Michael F. and Wolfe, Ralph S.(1993) 'F430-Dependent Biocatalysis in Methanogenic Archaebacteria', Comments on Inorganic Chemistry, 15: 1, 1 — 26
To link to this Article: DOI: 10.1080/02603599308035833
URL: http://dx.doi.org/10.1080/02603599308035833

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Downloaded At: 12:51 15 January 2011

F430-Dependent Biocatalysis in Methanogenic Archaebacteria

HOSHIK WON
Department of Chemistry, Emory University, Atlanta, Georgia 30322

KARL D. OLSON
Department of Microbiology, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801

MICHAEL F. SUMMERS
Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore, Maryland 21228

RALPH S. WOLFE
Department of Microbiology, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801

Received December 28, 1992

Coenzyme F430 is a Ni(II)-containing corphinoid which was first isolated from the cells of methanogenic archaebacteria in 1978. The cofactor F430 is responsible for the conversion of CO_2 into CH_4 in the methanogenic process. Starting from the first structural studies of coenzyme F430 with application of NMR and biosynthetic methods, many papers have been published to discuss the structural features as well as the biocatalytic role of F430. However, a precise understanding of the mechanism of F430-dependent catalysis is lacking. With a focus on what is known

Comments Inorg. Chem.
1993, Vol. 15, No. 1, pp. 1–26
Reprints available directly from the publisher
Photocopying permitted by license only

© 1993 Gordon and Breach,
Science Publishers SA
Printed in Malaysia

about the F430-dependent biocatalysis in methanogens, the results of physical and chemical studies of F430 are presented. In addition, functions of novel enzymes involved in the methanogenic process are discussed.

Key Words: *coenzyme F430, Ni(II)-containing corphinoid, methanogens*

Abbreviations

MFR: Methanofuran, 4-[N-(4,5,7-tricarboxyheptanoyl-γ-L-glutamyl-γ-L-glutamyl-)-p-(β-aminoethyl)phenoxymethyl]-2-(aminomethyl)furan
H_4MPT: Tetrahydromethanopterin, 7-methylopterin (2-amino-4-hydroxy-7-methylpteridine)
Coenzyme M (HS-CoM): 2-Mercaptoethanesulfonic acid
HS-HTP: 7-(Mercaptoheptanoyl)-L-threonine-O^3-phosphate
F420: Deazaflavin, (N-(N)-L-Lactyl-γ-L-glutamyl)-L-glutamic acid phosphodiester of 7,8-didemethyl-8-hydroxy-5-deazariboflavin 5′-phosphate)
F430: Cofactor, nickel(II)-containing corphinoid
F430M: Pentamethylated ester form of coenzyme F430
NMR: Nuclear Magnetic Resonance
TFE-d_3: Deuterated trifluoroethanol
NOESY: Nuclear Overhauser Effect Spectroscopy
HOHAHA: Homonuclear Hartmann–Hahn spectroscopy
HMQC: Heteronuclear Multiple Quantum Coherence spectroscopy
HMBC: ^{1}H-detected Heteronuclear Multiple Bond Coherence spectroscopy
DG: Distance Geometry
XAS: X-ray Absorption Spectroscopy
EXAFS: Extended X-ray Absorption Fine Structure
CD: Circular Dichroism
MCD: Magnetic Circular Dichroism
RR: Resonance Raman spectroscopy
EPR: Electron Paramagnetic Resonance
RP-HPLC: Reverse-Phase High Pressure Liquid Chromatography

Downloaded At: 12:51 15 January 2011

I. METHANOGENS AND THEIR NOVEL COENZYMES

Methanogens are unique microbes occupying a plethora of anaerobic habitats.[1,2] Methanogenic archaebacteria are distinguished from eucaryotes and eubacteria on the basis of homologies of partial sequences of their 16S ribosomal ribonucleic acid (rRNA). These organisms can be isolated from virtually every habitat in which anaerobic biodegradation of organic compounds occurs, including fresh water and marine sediments, digestive and intestinal tracts of animals, and anaereobic waste digesters.[2] They are capable of utilizing only a limited number of substrates (CO_2, formate, methanol, methylamines, isopropanol, ethanol and acetate) as carbon

and energy sources. Most methanogens can grow on H_2 and CO_2 by a complex set of reactions. The overall balanced equation and the thermodynamic free energy change[3] are given in reactions (1):

$$CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O$$
$$\Delta G^{o\prime} = -131 \text{ kJ/mol } CH_4 \qquad (1)$$

The obligate biosynthesis of methane is the signature of these microbes; in the entire biological ecosystem, only methanogens are known to couple growth to the synthesis of methane. These organisms are very sensitive to dioxygen. For this reason, they were difficult to work with until improved methods of culture were developed.[4]

At least six novel coenzymes (including F430) are involved in the biocatalytic conversion of CO_2 to CH_4.[5] The eight-electron reduction is accomplished by successively reducing the C_1 group as it is passed from coenzyme to coenzyme in a bucket-brigade fashion as shown in Fig. 1.[6] Figure 2 shows the complete structures of the five novel coenzymes involved in methanogenesis. Biochemically, the best-studied organism among the methanogens is *Methanobacterium thermoautotrophicum*. To acquaint the reader with the biochemistry of methanogens, a brief discussion of the conversion of CO_2 to CH_4 is described here.

Downloaded At: 12:51 15 January 2011

Formyl-Level of Oxidation

The first two-electron reduction involves the conversion of CO_2 to the formyl-level of oxidation. This formyl group is bound to the primary amine of methanofuran; thus, the chain of reactions starts with the formation of an N-substituted carbamate of CO_2 and *methanofuran (MFR)*, followed by its reduction to formyl-MFR. The structure of MFR has been elucidated and is 4-[N-(4,5,7-tricarboxyheptanoyl-γ-L-glutamyl-γ-L-glutamyl-)-p-(β-aminoethyl)phenoxymethyl]-2-(aminomethyl)furan.[7] The reaction is catalyzed by formyl-MFR dehydrogenase, which is a molybdenum–iron–sulfur protein.[8] The enzymology of this reaction is not well understood and involves the role of molybdopterins.[9] Like other

Downloaded At: 12:51 15 January 2011

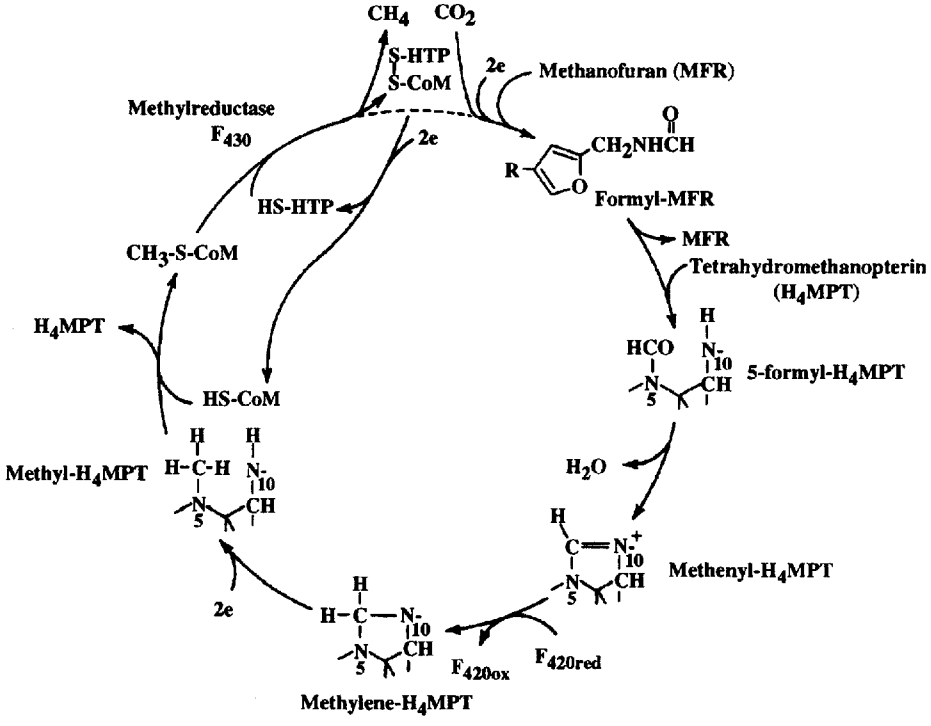


FIGURE 1 Cyclic represention of the methanogenic pathway of CO_2 to CH_4.

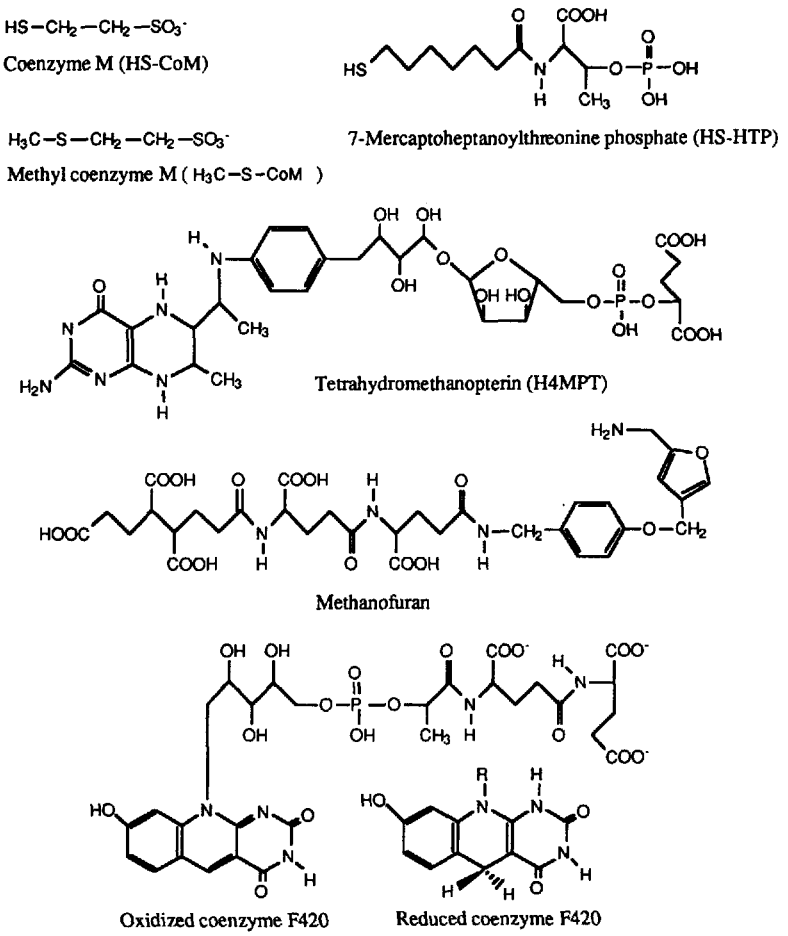


FIGURE 2 Molecular structures of novel coenzymes found in methanogens.

cofactors, methanofuran appears in the polyglutamated form in *Methanosarcina barkerii*.[10,11]

Methylene and Methyl Levels of Oxidation

The formyl-group is then passed to the second novel coenzyme, *tetrahydromethanopterin (H_4MPT)*, a folic acid analogue. This

Downloaded At: 12:51 15 January 2011

molecule is a 7-methylpterin (2-amino-4-hydroxy-7-methylpteridine). It is derivatized with the formyl, methenyl, methylene, or methyl C_1 groups linked to either or both the N-5 and N-10 positions of the pterin moiety. In this series of reactions the formyl group is transferred to H_4MPT by the enzyme formylmethanofuran: tetrahydromethanopterin formyltransferase. The methenyl group of methenyl-H_4MPT is reduced to a methylene group by the enzyme, methylenetetrahydromethanopterin:coenzyme F420 oxidoreductase. The third novel coenzyme, *F420*, a deazaflavin (N-(N)-L-lactyl-γ-L-glutamyl)-L-glutamic acid phosphodiester of 7,8-didemethyl-8-hydroxy-5-deazariboflavin 5′-phosphate), provides the electrons for this reaction.[12,13] Coenzyme F420 is an obligate two-electron donor and possesses a midpoint potential of −370 mV (vs. NHE).[14] It was named because of its intense absorption at 420 nm.

The enzyme that catalyzes the next two-electron reduction (from the methylene-level to the methyl-level of oxidation) has recently been purified and shown to be a methyltetrahydromethanopterin:coenzyme F420 oxidoreductase. Once again, reduced F420 provides the electrons for this reaction.[15,16]

The transfer of the methyl group to the fourth novel coenzyme, *HS-CoM* is not well understood, but evidence suggests that a carbamide enzyme, which also may be a sodium pump, is involved in methyl transfer.[18]

Downloaded At: 12:51 15 January 2011

Conversion of CH_3-S-CoM to CH_4

The final step of methanogenesis is probably the most complex of all the reactions. There are no fewer than three novel coenzymes involved in this reaction, indicating that this was probably not a trivial reaction for nature to invent. It is also perhaps the most interesting step from a chemical perspective, since it is the methane yielding reaction—a reaction unique in the biological world. This reaction is the reductive demethylation of 2-(methylthio)ethanesulfonic acid (CH_3-S-CoM) with a reducing equivalent from the fifth novel coenzyme, *7-(mercaptohepatanoyl)-L-threonine-O^3-phosphate (HS-HTP)*. The products of this reaction are methane and the unsymmetrical disulfide of 2-mercaptoethane-

sulfonic acid (HS-CoM) and HS-HTP (CoM-S-S-HTP)[11,12] The balanced equation is presented in reaction (2):

$$CH_3\text{-S-CoM} + \text{HS-HTP} \rightarrow CH_4 + \text{CoM-S-S-HTP} \quad (2)$$

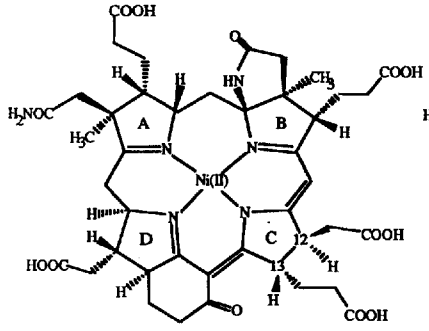
A product of the CH_3-S-CoM reductase reaction, CoM-S-S-HTP, is involved in the fixation (an activation) of CO_2 to form formyl-MFR.[18a] That is, the terminal step is coupled to the initial step of methanogenesis. The mechanism of this activation is not known. For this reason, the above described series of reductions is presented in cyclic form in Fig. 1. Only partial structures (i.e., the reactive end of the molecules) are shown.

The yellow enzyme that catalyzes the methane-yielding reaction is the 2-(methylthio)ethane-sulfonic acid reductase (CH_3-S-CoM reductase). The protein is highly acidic (acidic amino acids outnumber basic ones by a factor of 2:1). In *M. thermoautotrophicum* (strain ΔH), the enzymatic complex which reductively demethylates the substrate has been shown to be composed of a complex set of proteins, including the reductase, A1, A2, A3a, and A3b.[18b] Component A1 may be involved in the transfer of electrons from H_2 to CoM-S-S-HTP (a product of the methylreductase reaction); A2 may catalyze an allosteric modification of the reductase (producing a more positive midpoint potential); A3a is a large iron–sulfur protein involved in electron transfer to the reductase. A3b is required when H_2 and HS-HTP are provided as the dual source of electrons. Only the CH_3-S-CoM reductase and A2 have been purified to homogeneity.[6] Another difficulty with this system is that there are no individual assays for each component. Only through laborious cross-titration experiments can the system be reconstituted. In contrast, the CH_3-S-CoM reductase from strain ΔH has been purified in highly active form and does not require the A components for activation.[17] Bound to this enzyme (non-covalently) is the sixth novel coenzyme, *F430*. The stoichiometry is two moles of F430 per mole of enzyme.

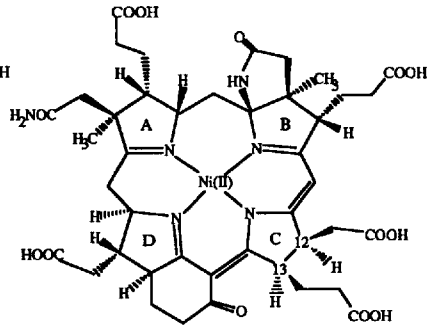
Native coenzyme F430 is a tetrapyrrole (tetrahydrocorphin) containing the transition metal Ni as shown in Fig. 3. It also has a lactam ring fused to the B ring and a unique cyclohexanone fused to the D ring of the macrocycle. The CH_3-S-CoM reductase has a molecular weight of about 300,000. It consists of three different

Downloaded At: 12:51 15 January 2011

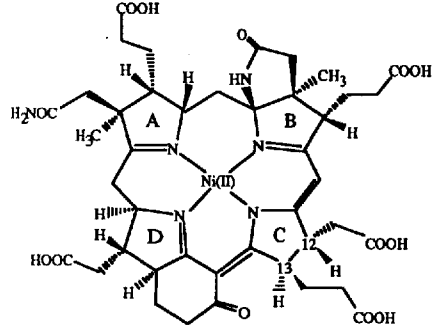
Downloaded At: 12:51 15 January 2011



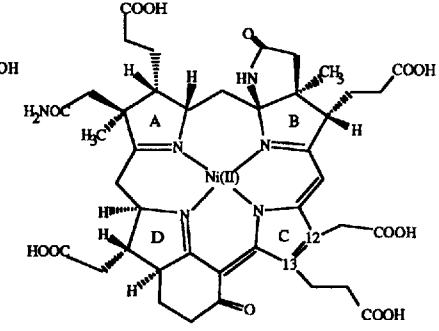
Native Coenzyme F430



13-Monoepimeric F430



12,13-Diepimeric F430



12,13-Didehydro F430

FIGURE 3 Structure of native coenzyme F430 and its analogues.

subunits with apparent molecular weights [in *Methanobacterium thermoautotrophicum* (strain ΔH)] of 68,000, 45,000, and 38,500 daltons. The stoichiometry is therefore α_2, β_2, γ_2.[18c] Besides F430, the isolated enzyme is found to contain two tightly bound equivalents of HS-CoM.[17] HS-HTP is also bound to the enzyme; a stoichiometry of two is expected.[19]

Jean LeGall first noticed factor F430 in extracts of *Methanobacterium thermoautotrophicum* (due to its strong absorption at 430 nm). The isolation of coenzyme F430 was first reported by R. P. Gunsalus and R. S. Wolfe in 1978.[20] Within two years, two laboratories independently discovered that F430 contains nickel. Soon afterward, Thauer and co-workers found that 8 mol of either succinate[21] or δ-aminolevulinic acid[22] was incorporated per mole of F430 (by the radioactive precursors). This was strong evidence that the chromophore was a tetrapyrrole. δ-Aminolevulinic acid is a biosynthetic precursor of all natural prophinoids and corrins.[23,24] The biosynthetic pathways of several known tetrapyrroles are shown in Fig. 4. The chemical structure of F430 was established in a collaborative effort by the laboratories of Eschenmoser and Thauer in 1982.[25] In addition to classical 1D ^1H and ^{13}C NMR studies (e.g., nuclear Overhauser effect spectroscopy), a wide array of chemical and instrumental methods was used to deduce

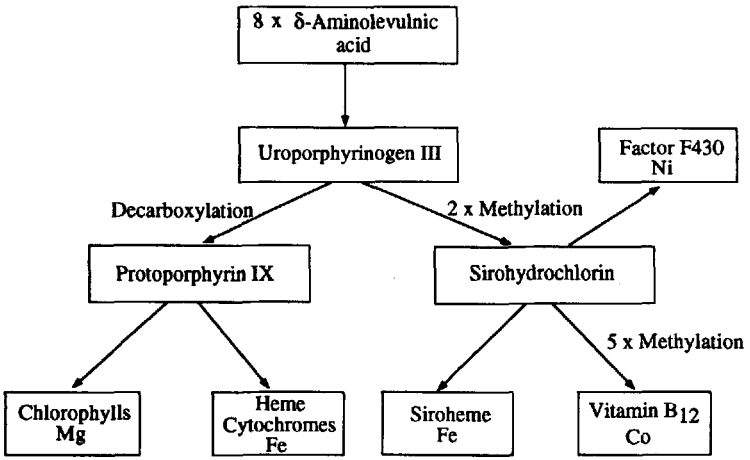


FIGURE 4 Biosynthetic pathways of several known tetrapyrroles.

Downloaded At: 12:51 15 January 2011

theprimary structure, including, UV/Vis spectroscopy, circular dichroism, I.R. and mass spectroscopy and ozonolytic degradation. Ten of the eleven macrocyclic stereochemistries were determined. The chirality assignment of C17 on the D-ring could not be made due to severe overlap in the 1D NMR spectrum.

In 1984, Robert Hausinger showed that there are two pools of F430 found intracellularly in methanogens. One pool is bound (non-covalently) to the enzyme, the CH_3-S-CoM reductase, and another pool can be found unbound (i.e., "free") in the cytosol.[26] Thauer and co-workers were able to show that when the methanogenic cells were grown under Ni-limiting conditions, F430 existed mainly in an enzyme-bound form. All methanogenic bacteria investigated incorporate nickel in coenzyme F430.[21]

Once extracted from the holoenzyme (by the relatively mild conditions of salt and ethanol) native F430 undergoes complex chemistry. The cofactor is thermal and oxygen sensitive. In the presence of heat, native F430 undergoes epimerization of the acid side chain at β-carbon positions 12 and 13 (see Fig. 3). The 12,13-diepimer is the product of this epimerization. The intermediate step is a single epimerization at the chiral position of C13. The native coenzyme is also O_2-sensitive and is slowly oxidized to the more stable 12,13-didehydro F430 (F560) in which a double bond is formed between β-carbons 12 and 13 of the C ring.[27] Figure 3 shows the structures of native F430 and its analogues. Only the native configuration is believed to have biological significance, but this certainly has not been proven. It is also not known if an epimerase exists in methanogens to convert the diepimer back to the native stereochemical configuration. F430 exists as a hydrocorphinoid, i.e., a porphinoid C-N ligand skeleton combined with a corrinoid chromophore system.[28–30] In addition, it also exhibits the most reduced macrocycle known to exist in the biological world.[27] Native coenzyme F430 and its analogues can be separated by various types of anion exchange chromatography and RP-HPLC.[31,32]

Nickel has unique coordination and redox properties; it exhibits oxidation states from -1 to $+3$. Nickel-containing macrocycles are extremely rare in the biological world; only two are known to exist, one is F430 and the other is tunichlorin found in the marine tunicate *Tridemnum solidum*.[33]

Downloaded At: 12:51 15 January 2011

II. STUDIES OF COENZYME F430

Since the discovery of coenzyme F430, numerous spectroscopic methods have been carried out in order to understand the F430-dependent function in methanogens. Studies of F430-chemistry have focused on the understanding of coordination modes, structural features and the mechanism of F430-dependent catalysis. As discussed in the previous section, cofactor F430 is responsible for the conversion of CH_3-S-CoM into methane by using a reducing equivalent from HS-HTP. The production of methane is an energy-yielding process in methanogens.[4–7]

The changes of nickel oxidation state (Ni(II)-Ni(III) or Ni(II)-Ni(I)) of F430 may be involved in this biocatalytic process.[34,35] Therefore the systematic characterization of the electrochemical properties of these metal ions is essential to an understanding of the terminal step of methanogenesis. Why has nature invented a highly saturated corphinoid to accommodate the nickel ion rather than other metal ions? A Ni(I)-EPR signal in intact cells has been observed, suggesting that Ni(II) in the macrocycle center has been reduced.[36,37] The presence of Ni(I) species in the CH_3-S-CoM reductase has increased curiosity about the physiological role of Ni(II) associated with the highly reduced macrocycle and the active site of the protein-bound F430.

Native coenzyme F430 is quite polar because the corphinoid has five carboxylic groups as well as the electrophilic Ni(II) macrocyclic center, so F430 dissolves well in polar solvents like water and methanol. Solvation gives rise to the paramagnetic properties of two unpaired electrons occupied in nickel d_{z^2} and $d_{x^2-y^2}$ orbitals. Interestingly, until recently this paramagnetism hampered the detailed structural analysis of native F430 by NMR.[38,39] Native F430 exhibits partial diamagnetic properties in non-nucleophilic trifluoroethanol (TFE-d_3) solvent, in which it does not form an octahedral environment. However, the first structural studies by NMR could not be made due to the severe broadening of the NMR signals.[40] In addition, structural studies by X-ray crystallography were precluded due to the difficulties, in part, in obtaining suitable crystals. The paramagnetic properties of F430 as well as the difficulties in crystallizing native F430 have led to numerous F430-model studies and other synthetic approaches to understand F430-chemistry. In

Downloaded At: 12:51 15 January 2011

this section, the progress of previous structural studies of coenzyme F430 is discussed.

NMR Studies

The first structural studies of coenzyme F430 involved the application of NMR and biosynthetic methods.[25] The native free acid is quite polar (soluble mainly in H_2O and MeOH), is paramagnetic in these nucleophilic solvents, and is therefore not amenable to high resolution NMR studies.[40] The pentamethyl ester derivative of F430, F430M, however, is soluble in non-coordinating organic solvents (e.g., CD_2Cl_2) and is diamagnetic under these conditions. The ^{1}H-NMR spectroscopic studies of F430M were made with classical one-dimensional (1D) ^{1}H-NMR NOE difference spectroscopy and employed biosynthetically labeled ^{13}C precursors of F430. Partial ^{1}H- and ^{13}C-NMR signal assignments of F430M were made by using the stereochemistries and NMR signal assignments of several known model complexes.[25] In these studies, the stereochemical assignments were made for 10 of the 11 chiral corphin macrocyclic ring carbons. Partial ^{13}C-NMR assignments were made for the native form of F430 (free acid) in deuterated trifluoroethanol (TFE) solution based upon the comparison of the ^{13}C-NMR spectrum of F430M.

Although paramagnetic line broadening precluded NMR studies using aqueous solutions, ^{1}H NMR signals of the native form of coenzyme F430 obtained using TFE-d_3 as the solvent at high magnetic field strength were sufficiently narrow to allow detailed investigations with recently developed 2D NMR techniques, including homonuclear and ^{1}H-^{13}C heteronuclear correlation experiments.[32] Complete ^{1}H-NMR signal assignments for all non-exchangeable protons and unambigous ^{13}C-NMR signal assignments were accomplished for native F430 and 12,13-diepimeric F430.[32,39]

From these studies, which included application of HMBC spectra, the primary structure of native F430 was confirmed.[32] Interestingly, the stereochemistry of the C17 carbon could not be determined due to severe signal overlap,[25] and the relative stereochemical assignments for C18 and C19 were made on the basis of weaker NMR and chemical data and were considered to be tentative.[32] The C17-C18-C19 assignments were determined to

Downloaded At: 12:51 15 January 2011

be either **R-R-S** (original assignments) or **S-S-R** (reverse assignments).[32] The results of stereochemical analysis that employed 2D NOE back-calculations for the F430 model structures generated by distance geometry (DG) computations indicated that the reverse structure, if it existed, should contain internal strain.[41] The solution-state structure of 12,13-diepimeric F430 was recently made using 2D NMR methods in combination with distance geometry and Overhauser effect back-calculation methods. The superpositions of DG structures with the X-ray structure of the pentamethyl ester form of 12,13-diepimeric F430 indicated good structural agreement with an average RMS deviation for corphin macrocyclic atoms of 0.28 Å.[39]

In addition to the structural features and signal assignments by NMR, axial ligand binding studies have recently been accomplished.[42] The oxidation state of Ni(II)-F430M was sufficiently reduced to Ni(I) to accommodate the axial ligand CD_3. CH_3-F430M was proposed as an intermediate in the enzymatic process catalyzed by the CH_3-S-CoM reductase as shown in Fig. 5. In these studies, F430M could be chemically reduced in a non-coordinating solvent to the Ni(I)F430M species without adversely affecting the corphi-

Downloaded At: 12:51 15 January 2011

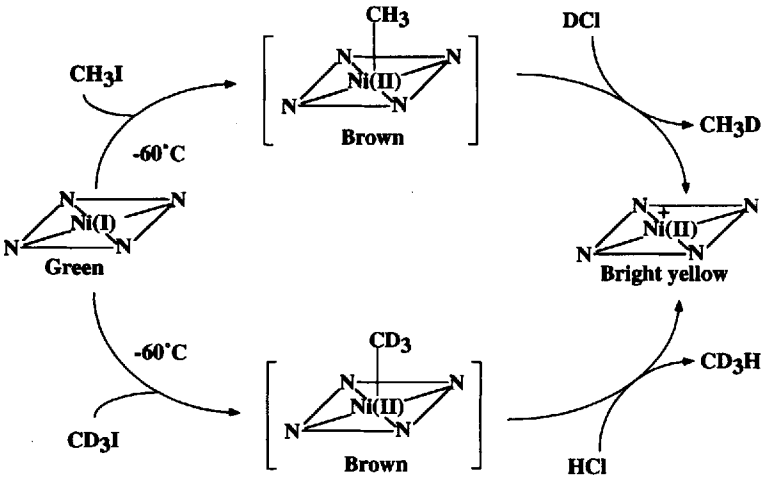


FIGURE 5 Scheme representing the formation of a methylnickel(II) derivative of coenzyme F430 proposed as an intermediate in the enzymatic process.

noid macrocycle. The addition of CH_3X (X = halides) to solutions of Ni(I)F430M at low temperature ($-60°C$) produces methane via a possible complex formation, CH_3-Ni(II)F430M. This complex is relatively stable at low temperatures, and no methane is produced under these conditions. However, methane is evolved upon warming the solution (with a concomitant change in color). This is an indication that the mechanistic process of coenzyme F430 may undergo an oxidoreduction process via Ni(I) in the final step of the conversion of CH_3-S-CoM into methane.

UV/Vis and CD Studies

The isolation of F430 from the CH_3-S-CoM reductase leads to the major free species of native F430 (C12, **S**; C13, **S**), the 12,13-diepimeric F430 (C12, **R**; C13, **R**), the 13-monoepimeric F430 (C12, **S**; C13, **R**) and the 12,13-didehydro F430 (an oxidation product, F560)[25] (see Fig. 3). However, it is not known whether the two stereoisomers and F560 are physiologically important.[43] F560 exhibits a purple color due to the extended conjugation of the macrocyclic ring, whereas the native coenzyme F430 and its isomers exhibit a yellow color. The circular dichroism (CD) spectrum of the 12,13-diepimeric F430 is about two or three times as intense as that of the native form of F430 throughout the visible region. The stereochemistries of two C12 and C13 carbons could easily be made by comparing the relative stereochemistries obtained from the ozonolysis product of F430M, vitamin B_{12} and sirohydrochlorin.[25]

CD and UV/Vis spectroscopies could also easily distinguish the diamagnetic, ruffled-macrocyclic 12,13-diepimeric F430 from the paramagnetic, planar-macrocyclic native F430 as shown in Fig. 6. For example, the paramagnetism of the native F430 giving rise to the pseudo-octahedral Ni(II) environment could be observed by the magnetic circular dichroism (MCD) spectrum.[44] The UV/Vis absorption peak near 430 nm is slightly blue shifted (by 10 nm) in the six-coordinated native form of F430 compared to that of the 12,13-diepimeric F430. The UV/Vis peak is flanked by two weak absorptions at ~500 nm and ~340 nm (tentatively assigned as d-d bands) only in the spectrum of 12,13-diepimeric F430[29,30,43] (see Fig. 6). The structural studies completed by NMR, several

Downloaded At: 12:51 15 January 2011

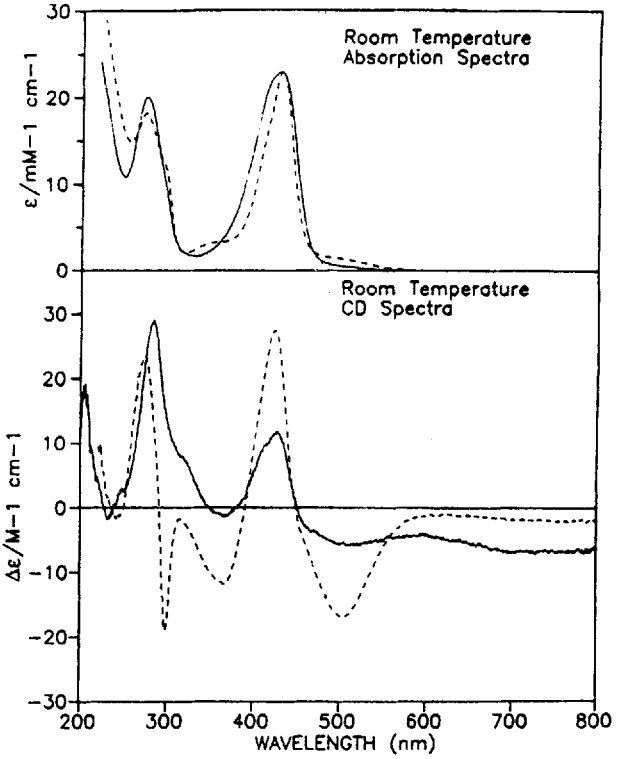


FIGURE 6 UV/Vis and CD absorption spectra for native (——) and 12,13-di-epimeric F430 (---).

Downloaded At: 12:51 15 January 2011

biosynthetic-labeling methods, UV/Vis and CD spectroscopies were used to deduce the entire molecular skeleton for native F430 and its isomers.

XAS and EXAFS Studies

Structural investigations by X-ray absorption (XAS) and Extended X-ray Absorption Fine Structure (EXAFS) spectroscopies were used to determine the Ni-ligand bond distances as well as the axial ligation modes of F430 in aqueous solution, in nonaqueous solution and in the CH_3-S-CoM reductase holoenzyme. The coordination geometries and numbers of F430 were assigned by comparing a

large number of XAS results for Ni(II) model compounds.[45,46] For example, the 12,13-diepimeric F430 exhibits a nickel K-edge X-ray absorption spectrum with a transition at ~8336 eV, which is a signature of square-planar coordination as shown in Fig. 7. The six-coordinate native F430 (presumably with two axially bound water molecules) is predominant at temperatures below 250 K. However, the four-coordinate form of native F430 is the predominant species in TFE and 2-mercaptoethanol solvents.[45]

EXAFS experiments indicated that native F430, extracted from the CH_3-S-CoM reductase, is a six-coordinate pseudo-octahedron exhibiting long Ni-N bond distances of ~2.1 Å with two axial ligands (in aqueous solution).[45] The MCD spectra obtained for both free and protein-bound F430 are highly similar, and it was suggested that the axial ligation may occur by either water ligands or by other O-donor ligands with binding strengths similar to that of water. Analysis of the low temperature EXAFS data of the 12,13-diepimeric F430 in aqueous solution yields a Ni-N distance of 1.89 Å.[45,47] By contrast, native F430 exhibits a sharp absorption spectrum typical of octahedral Ni(II) complexes at low temperature. XAS and EXAFS studies showed that F560 is also in a four-coordinate geometry with Ni-N bond distances of 1.9 Å.

The macrocyclic core-hole radius of native F430 is larger than that of the 12,13-diepimeric F430 by 0.2 Å. The probable reason for this difference was explained by the ability of the F430 macrocycle to flex between a nearly planar (in native F430) to a highly S4-ruffled conformation (in the diepimer), a behavior which is observed in Ni(II) pyrocorphin model compounds.[45,46,48-51] This flexibility is afforded by the highly reduced nature of the F430 tetrapyrrole. The expansion of the Ni(II) coordination sphere and the increase in coordination number are correlated with a Ni(II) spin-state change. Native F430 exhibits the paramagnetic ($S = 1$) ground state expected for pseudo-octahedral Ni(II), whereas the 12,13-diepimeric F430 is diamagnetic.[43] The Fourier transform (FT) data of EXAFS indicated that the Ni-N distances of F430 observed in the CH_3-S-CoM reductase are longer than those of protein-free F430. In addition, the axial ligand-binding studies with cyanide, pyridine, and 1-methylimidazole ligands indicated that these axially coordinating ligands bind more tightly (~40-fold) to the native F430 compared to the 12,13-diepi-F430. These results suggest that

Downloaded At: 12:51 15 January 2011

Downloaded At: 12:51 15 January 2011

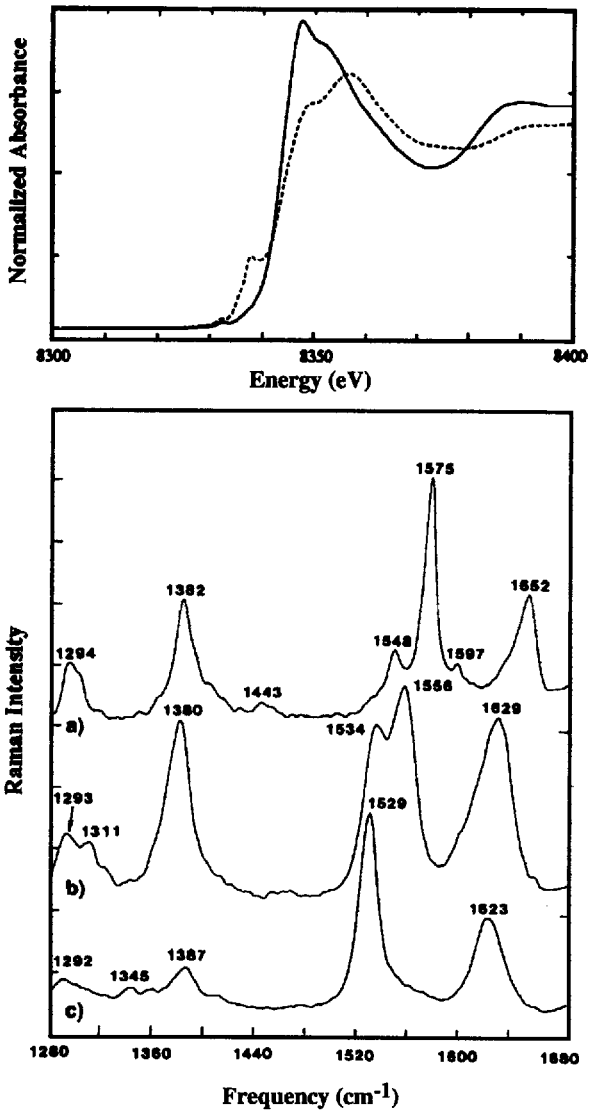


FIGURE 7 Resonance Raman spectra of methyl reductase (a), native F430 (b), and the 12,13-diepimeric F430 (c); (Bottom) Nickel K-edge X-ray absorption spectra of native F430 (——) and 12,13-diepimeric F430 (---) in aqueous solution (Top).

the protein control of planarity may affect the expansion of corphin ring and axial ligand affinity.[46] X-ray absorption spectroscopic data indicate that the Ni in native F430 (free and bound to the CH_3-S-CoM reductase) has a Ni-N bond distance of 2.10 ± 0.02 Å. The 12,13-diepimer, in contrast, has a Ni-N bond length of 1.89 ± 0.02 Å.[43,45,46] The shorter bond distances in the diepimer (i.e., in the axially uncoordinated form of F430) are indicative of a ruffled macrocycle. The thermal stability of the diepimer over the native form can be rationalized by considering this ruffling, the conformation of which is known as the W-conformation, so named because of the shape of the cylindrical projection.[49-51]

There appear to be steric repulsions between the carbonyl group on the saturated carbocyclic ring attached to the D-ring of the macrocycle and the C13 propionic acid side chain. This repulsion forces this side chain to be quasi-axial. It is now known that the nickel in F430 is pseudo-octahedrally coordinated in the holoenzyme[45]; the two axial ligands are believed to be supplied by the protein. The identity of the ligands is not known. All known crystal structures of nickel(II) complexes with hydroporphinoid ligands have a characteristic S_4 deformation of the ligand periphery. It is believed that the ruffling is the consequence of a contraction of the coordination hole of the hydroporphinoid ligand induced by the relatively small nickel(II) ion which tries to reach saturation of electrophilicity.[49]

Downloaded At: 12:51 15 January 2011

Resonance Raman (RR) Studies

The possible coordination numbers and geometries of F430 were also predicted by comparing the Raman separations in the high frequency region (~1500–1650 cm^{-1}) which represent the specific ligation modes of F430 and model complexes.[28,29] The frequency values of Raman separation for nickel corphinoid nickel complexes that are similar to F430 structure were as follows: 93 cm^{-1}, 4-coordination; 80 cm^{-1}, 5-coordination; and 71 cm^{-1}, 6-coordination.

The RR studies for the well-purified forms of F430 in aqueous solution indicate that the spectra of native F430 (a mixture of four- and six-coordination) are clearly different from those of the 12,13-diepimeric F430.[28,29] The Raman separation of 12,13-diepimeric

F430 (94 cm^{-1}) is consistent with the XAS and EXAFS results indicating that the diepimeric F430 is a four-coordinate, square-planar structure with Ni-N distances of 1.9 Å. However, the 77 cm^{-1} separation value of the CH_3-S-CoM reductase is considerably different from that of either native F430 or 12,13-diepimeric F430 as shown in Fig. 7.[29] Although the spectrum obtained for the bis-pyridine adduct comes closest to reproducing the CH_3-S-CoM reductase spectrum, no six-coordinate F430 complexes that resolve this discrepancy in the high frequencies were reported. It was suggested that a six-coordinate cofactor F430 with novel ligation is responsible for the anomalous holoenzyme spectrum.

In axial ligand-binding studies, the RR spectra of native F430 were dramatically changed in the presence of axial ligands in aqueous solution. The fingerprints of the four-coordinate form of F430 become the characteristic six-coordinate complexes (by exhibiting a 70 cm^{-1} separation of the two high-frequency bands) in the presence of pyridine, 1-methylimidazole, and cyanide ion.[29,43]

Downloaded At: 12:51 15 January 2011

X-Ray Crystallographic Studies

Although the structure of native F430 has yet to be determined, detailed structural features, including the degree of macrocyclic ring-puckering and the bond lengths of Ni-N, have been obtained from X-ray crystallographic studies of F430-model complexes. Extensive X-ray analyses for high-spin and low-spin hydrocorphinoid Ni(II) F430-model complexes correctly predicted the nature of F430.[48–51] Several diamagnetic Ni(II)-N_4 corphinoid complexes exhibit a common saddle-shaped conformation with S4 symmetry.[49] This deformation is believed to be dependent on the degree of the saturation of porphyrin rings and the electrophilicity of Ni(II) ion. The electrophilicity of the nickel ion in corrin and hydrocorphin complexes lies in the core-hole of the ring in terms of ruffled-ligand conformation and strain energy due to this ruffling.[50]

The structure of 12,13-diepimeric F430M (pentamethyl ester derivative 12,13-diepimeric F430) was recently obtained by X-ray crystallographic methods. As predicted in extensive F430-model studies, the molecular consitution exhibits a ruffled-S4 geometry

with Ni-N bond distances of 1.85 Å (cf. 1.89 Å predicted from EXAFS studies).[30] The original stereochemical assignment of the C17 chiral carbon was not made due to severe ^{1}H-NMR signal overlap and the relative stereochemical assignments for C18 and C19 were made on the basis of weaker NMR and chemical data. In addition, C17-C18-C19 assignments were determined to be either **R-R-S** (original assignments) or **S-S-R** (reverse assignments)[32] on the basis of 2D-NOESY peak intensities.

However, the X-ray crystallographic results of F430M have unambiguously reassigned the stereochemical assignments of C17-C18-C19 (**R-S-S**) to be C17-C18-C19 (**S-S-R**).[39] In addition, F430 has a geometrically unique lactam ring moiety compared to the porphyrin and corrin complexes (e.g., coenzyme B_{12}). Interestingly, recent structural studies show that the lactam ring amide proton is located on top of the corphine macrocyclic nickel center.[39] The free carboxylic acids of F430 and the amide proton of the lactam ring are believed to be important in the specific location of α-subunit of CH_3-S-CoM reductase protein and in putative substrate-binding.

Downloaded At: 12:51 15 January 2011

EPR Studies of F430

The electron paramagnetic resonance (EPR) signals were observed for Ni(I)-species in whole cell preparations of *M. thermoautotrophicum* as shown in Fig. 8.[36,37] The EPR studies indicate that the coordination geometries of both the CH_3-S-CoM reductase and Ni(I)-F430M are tetrahedrally distorted forms.[34,35] The signals (S = 1/2) have been attributed to Ni(I) species containing an odd electron in the $d_{x^2-y^2}$ orbital. The Ni(I) species appears to be involved in methane production. The presence of multiple Ni(I) EPR signals in spectra of whole cell samples is believed to arise from the differences in axial coordination geometries. EPR studies provide a clue that the Ni(I) species may be involved in the F430-dependent biocatalysis. The observation of Ni(I) species in a series of catalytic studies with RX (R = alkane, X = halide) have shown that Ni(II) plays a catalytic role in the production of methane and other alkanes when Ni(II) is reduced to Ni(I) by using a reducing agent.[34,35]

Downloaded At: 12:51 15 January 2011

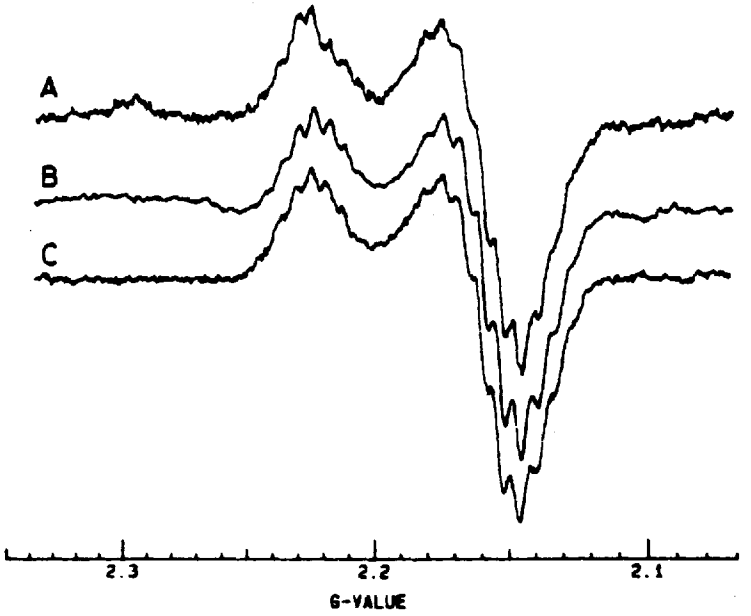


FIGURE 8 Electron paramagnetic resonance spectroscopy of native F430 and intact CH_3-S-CoM reductase. Intact cells kept under N_2 at 4°C (A); Cells disrupted by ultrasound in air and kept under H_2 for 18 hr at 4°C (B); Purified cofactor F430 (C).

III. REMAINING QUESTIONS AND CONCLUDING REMARKS

As described above, a wide array of spectroscopic characterizations and mechanistic studies of F430 have been performed to address the structural features as well as the exact biocatalytic role of coenzyme F430.

In the future, information regarding the interactions between F430 and potential axial ligands is necessary, since the catalytic mechanisms include either the formation of Ni-S (S-HTP) or Ni-CH_3 intermediates. The XAS/EXAFS data indicate that Ni is octahedrally coordinated in the resting-state form of the CH_3-S-CoM reductase, and as indicated in the previous section, both five- and six-coordinate Ni species have been implicated in various phases of the F430-dependent catalysis. In these respects, the axial ligand

binding studies are important from a biochemical perspective. The holoenzyme is very intolerant with respect to changes in the length of methylene carbons of either CH_3-S-CoM or HS-HTP, and HS-HTP cannot substitute as the methyl-group donor.[12,54] Structural studies of the relatively stable (at low temperature) organonickel complex (CH_3-F430M or RS-F430M) are necessary to determine if the structural deformations of F430 occur on the axial ligand binding. In addition, structural investigations of X-F430M complexes (X = monodentate ligands such as CN^-, CO, SCN^-, I^-, and RS- (R = alkyl) are important to gain insights into the stability and lability of axial bonds. The results of axial ligand binding studies can determine if the direction of ligand-binding is α-site (above the corphine macrocyclic plane) or β-site (below the corphine macrocyclic plane) of the macrocyclic tetrapyrrole plane. Since resonance Raman data indicate that bis-axial bindings are formed (more readily for F430 than for 12,13-diepimeric F430), the structural comparisons of native and 12,13-diepimeric F430 may provide information that the different chemistries result from differences in steric interactions between the lactam group and the bound axial ligands.

Suitable crystals of the CH_3-S-CoM reductase have not yet been obtained for X-ray crystallography. In addition, because of the high molecular weight of the α-subunit (~68,000 daltons) of the CH_3-S-CoM reductase, it is not currently possible to approach the 3D structure determination by NMR. However, since XAS studies have shown that Ni(II) in the CH_3S-CoM reductase is octahedrally coordinated and paramagnetic, it may be possible to examine selectively the ^{1}H-NMR signals due to residues that are relatively close to the Ni center,[53] including those of proximal amino acid residues, substrates, and potential inhibitors.

The isolation of F430 from the CH_3-S-CoM reductase yields native F430 and its isomers, including 13-monoepimeric F430 and the thermodynamically stable 12,13-diepimeric F430.[25,35] Interestingly, H13 of native F430 was observed to be labile in TFE-d_3 due to deuterium exchange as shown in Fig. 9.[32] The 3-D structure of F430 indicates that the carbonyl group of the D-sugar ring slightly perturbs H13. This interaction may be important in epimeric formation. The changes in the oxidation state of Ni(II) due to axial ligand-binding or possible substrate binding provide other expla-

Downloaded At: 12:51 15 January 2011

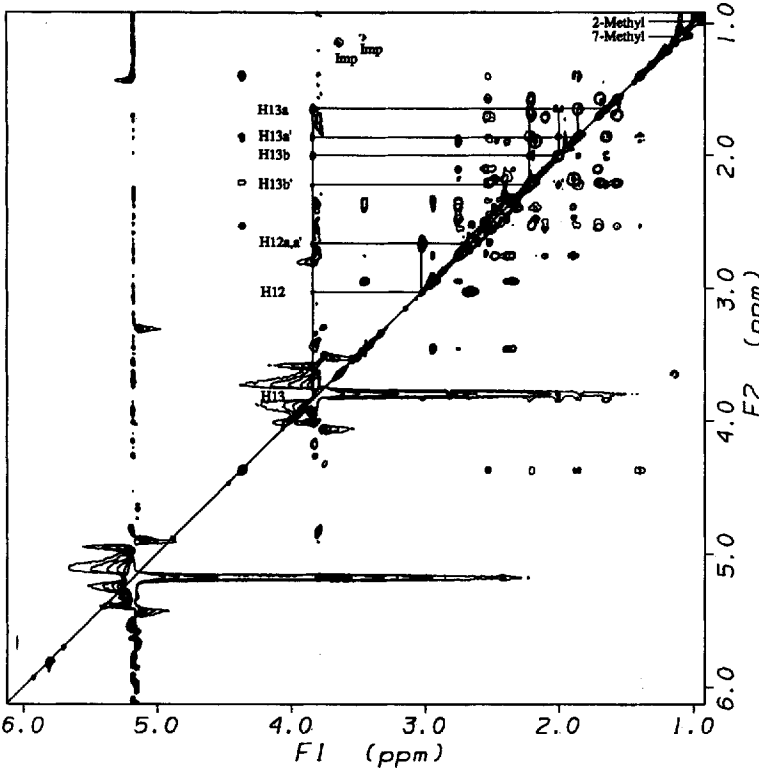


FIGURE 9 A portion of 2D HOHAHA spectrum of native coenzyme F430 immediately after dissolving into TFE-d_3 showing J-networks from H13 proton.

nations for epimerization. To date, no clear evidence has been reported regarding the physiological relevance of epimerization in methanogens.

Proposed mechanisms of F430-mediated demethylation include the substrate binding to nickel at some stage of the catalytic cycle. The finding of Ni(I)-EPR signals in intact cells as well as the isolation of Ni(I) and Ni(III) in F430M suggest that the mechanism includes a Ni(I) oxidation state of F430 via CH_3-Ni(II)-F430 or RS-Ni(II)-F430. A possible mechanism involves reduction of F430 to a Ni(I) species, followed by reaction of Ni(I)F430 with CH_3-S-CoM to yield the organonickel intermediate,[1] CH_3-Ni(II)F430, as shown in Fig. 10. Results of these related experi-

Downloaded At: 12:51 15 January 2011

ments provide a possible formation of an organonickel (square pyramidal) CH_3-Ni(II)F430M species. Although it is not known whether the CD_3 group is bound at the α- or β-site of macrocycle, the observation of CH_3-Ni(II)F430M strongly suggests that the methylreductase employs a CH_3-Ni(II)F430 intermediate in the reduction of a methyl group to methane.[7,34-37,55] A reductive activation of the CH_3-S-CoM reductase has been recently supported by the finding that the inactive enzyme can be partially activated in the presence of light.[55]

In summary, the history of F430-chemistry is relatively short compared to metallo-porphyrins and B_{12} chemistry. Historically, nickel has been considered to be biologically significant only because of its toxic effects, but it is now known as an essential requirement for many microorganisms. It is an important element, for example, in metabolic reactions of urease, hydrogen metabolism and acetogenesis.[1-3] Methanogenesis is large-scale biochemistry. Recent estimates indicate that near 400 million tons of methane enters the Earth's atmosphere annually, and 75% of this methane is generated by methanogens.[3,19] In pure scientific terms, the finding of coenzyme F430 is a milestone in the study of Ni(II)-containing corphinoid chemistry. To date, most research has focused mainly on the structural features and the coordination modes of F430, but detailed studies on the mechanism of F430-dependent biocatalysis are needed.

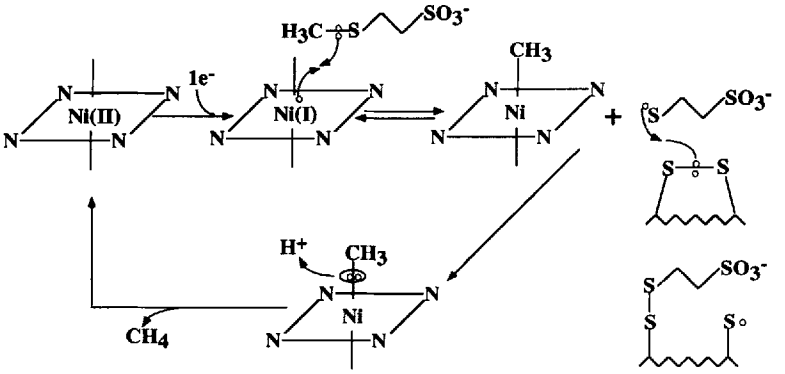


FIGURE 10 Possible mechanism for CH_3-S-CoM reductase involving one-electron reduction to a Ni(I) F430 species, formation of a methyl-nickel intermediate, and protonolytic decomposition.

Downloaded At: 12:51 15 January 2011

References

1. A. A. DiMarco, T. A. Bobik and R. S. Wolfe, Annu. Rev. Biochem. **59**, 355 (1990).
2. W. J. Jones, D. P. Nagle Jr. and W. B. Whitmann, Microbiol. Rev. **51**, 135 (1987).
3. R. K. Thauer, Biochim. Biophys. Acta **256**, 1018 (1990).
4. W. E. Balch and R. S. Wolfe, Appl. Environ. Micro. **32**, 781 (1976).
5. R. S. Wolfe, in *The Molecular Basis of Bacterial Metabolism* (Springer-Verlag, Berlin, 1990), pp. 1–12.
6. P. E. Rouvière and R. S. Wolfe, J. Biol. Chem. **263**, 7913 (1988).
7. J. A. Leigh, K. L. Rinehart Jr. and R. S. Wolfe, J. Am. Chem. Soc. **106**, 3636 (1984).
8. G. Börner, M. Karrasch and R. K. Thauer, FEBS Lett. **244**, 21 (1989).
9. G. Börner, M. Karrasch and R. K. Thauer, FEBS Lett. **290**, 31 (1991).
10. T. A. Bobik, M. I. Donnelly, K. L. Rinehart Jr. and R. S. Wolfe, Arch. Biochem. Biophys. **254**, 430 (1987).
11. T. A. Bobik, K. D. Olson, K. M. Noll and R. S. Wolfe, Biochem. Biophys. Res. Commun. **149**, 455 (1987).
12. J. Ellermann, R. Hedderich, R. Boecher and R. K. Thauer, Eur. J. Biochem. **172**, 669 (1987).
13. P. L. Hartzell, G. Zvilius, J. C. Escalante-Semerena and M. I. Donnelly, Biochem. Biophys. Res. Commun. **133**, 884 (1985).
14. F. Jacobson and C. Walsh, Biochemistry **23**, 979 (1984).
15. B. W. Brommelstroet, C. M. H. Hensgens, J. T. Keltjens, C. van der Drift and G. D. Vogel, J. Biol. Chem. **265**, 1852 (1992).
16. K. Ma and R. K. Thauer, Eur. J. Biochem. **191**, 187 (1990).
17. S. R. Rospert, R. Bocker, S. P. J. Albracht and R. K. Thauer, FEBS Lett. **291**, 371 (1991).
18. B. Becker, V. Muller and G. Gottschalk, FEMS Microbiol. Lett. **91**, 239 (1992).

18a. T. A. Bobik and R. S. Wolfe, Proc. Natl. Acad. Sci. USA **85**, 60 (1988).

18b. P. E. Rouviére and R. S. Wolfe, J. Bacteriol. **171**, 4556 (1989).

18c. W. L. Ellefson and R. S. Wolfe, J. Biol. Chem. **256**, 4259 (1981).

19. K. M. Noll and R. S. Wolfe, Biochem. Biophys. Res. Commun. **139**, 889 (1986).
20. R. P. Gunsalus and R. S. Wolfe, FEMS Microbiol. Lett. **3**, 191 (1978).
21. G. Diekert, U. Konheiser, K. Piechulla and R. K. Thauer, J. Bacteriol. **148**, 459 (1981).
22. R. Jaenchen, G. Diekert and R. K. Thauer, FEBS Lett. **130**, 133 (1981).
23. H. Gilles and R. K. Thauer, Eur. J. Biochem. **135**, 109 (1983).
24. A. R. Battersby, in *Porphyrins and Metalloporphyrins*, ed. K. M. Smith (Elsevier, Amsterdam, 1975), pp. 61–122.
25. A. Pfaltz, B. Jaun, A. Faessler, A. Eschenmoser, R. Jaenchen, H. H. Gilles, G. Diekert and R. K. Thauer, Helv. Chim. Acta **65**, 828 (1982).
26. R. P. Hausinger, W. H. Orme-Johnson and C. Walsh, Biochemistry **23**, 801 (1984).
27. A. Pfaltz, D. A. Livingston, B. Jaun, G. Diekert, R. K. Thauer and A. Eschenmoser, Helv. Chim. Acta **68**, 1338 (1985).
28. A. K. Shiemke, C. L. Hamilton and R. A. Scott, J. Biol. Chem. **263**, 5611 (1988).

Downloaded At: 12:51 15 January 2011

29. A. K. Shiemke, W. A. Kaplan, C. L. Hamilton, J. A. Shelnutt and R. A. Scott, J. Biol. Chem. **264**, 7276 (1989).
30. C. L. Hamilton, R. A. Scott and M. K. Johnson, J. Biol. Chem. **264**, 11605 (1989).
31. L. G. M. Gorris, C. van der Drift and G. D. Vogels, J. Microbiol. Methods **8**, 175 (1988).
32. H. Won, K. D. Olson, R. S. Wolfe and M. F. Summers, J. Am. Chem. Soc. **112**, 2178 (1990).
33. K. C. Bible, M. Buytendorp, P. D. Zierath and K. L. Rinehart, Proc. Natl. Acad. Sci. USA **85**, 4582 (1988).
34. B. Jaun and A. Pfaltz, J. Chem. Soc., Chem. Commun. 1327 (1986).
35. B. Jaun and A. Pfaltz, J. Chem. Soc., Chem. Commun. 293 (1988).
36. S. P. J. Albracht, D. Ankel-Fuchs, R. Bocher, J. Ellermann, J. Moll, J. W. van der Zwaan and R. K. Thauer, Biochim. Biophys. Acta **955**, 86 (1988).
37. S. P. J. Albracht, D. Ankel-Fuchs, J. W. van der Zwaan, R. D. Fontijn and R. K. Thauer, Biochim. Biophys. Acta **870**, 50 (1986).
38. G. Färber, W. Keller, C. Kratky, B. Jaun, A. Pfaltz, C. Spinner, A. Kobelt and A. Eschenmoser, Helv. Chim. Acta **74**, 697 (1991).
39. H. Won, K. D. Olson, R. S. Wolfe, D. R. Hare, C. Kratky and M. F. Summers, J. Am. Chem. Soc. **114**, 6880 (1992).
40. D. A. Livingston, A. Pfaltz, J. Schreiber, A. Eschenmoser, D. Ankel-Fuchs, J. Moll, R. Jaenchen and R. K. Thauer, Helv. Chim. Acta **67**, 334 (1984).
41. K. D. Olson, H. Won, R. S. Wolfe, D. R. Hare and M. F. Summers, J. Am. Chem. Soc. **112**, 5884 (1990).
42. S. Lin and B. Jaun, Helv. Chim. Acta **74**, 1725 (1992); *ibid.* **75**, 1479 (1992).
43. A. K. Shiemke, J. A. Shelnutt and R. A. Scott, J. Biol. Chem. **64**, 11236 (1989).
44. M. R. Cheesman, D. Ankel-Fuchs, R. K. Thauer and A. J. Thomson, Biochem. J. **260**, 613 (1989).
45. M. K. Eidness, R. J. Sullivan, J. R. Schwartz, P. L. Hartzell, R. S. Wolfe, A. Flank, S. P. Cramer and R. A. Scott, J. Am. Chem. Soc. **108**, 3120 (1986).
46. R. A. Scott, P. L. Hatzell, R. S. Wolfe, J. Legall and S. P. Cramer, *Frontiers in Bioinorganic Chemistry* (VCH, 1986), p. 15.
47. G. P. Diakun, B. Piggott, H. J. Tinton, D. Ankel-Fuchs and R. K. Thauer, Biochem. J. **232**, 281 (1985).
48. C. Kratky, C. Angst and J. E. Johansen, Angew. Chem. Int. Ed. Engl. **20**, 211 (1981).
49. C. Kratky, A. Fässler, A. Pfaltz, B. Kräutler, B. Jaun and A. Eschenmoser, J. Chem. Soc. Chem. Commun. 1368 (1984).
50. C. Kratky, R. Waditschatka, C. Angst, J. E. Johansen, J. C. Plaquevent, J. Schreiber and A. Eschenmoser, Helv. Chim. Acta **68**, 1312 (1985).
51. R. Waditschatka, C. Kratky, B. Jaun, J. Heinzer and A. Eschenmoser, J. Chem. Soc. Chem. Commun. 1604 (1985).
52. B. Jaun, Helv. Chim. Acta **73**, 2209 (1990).
53. J. A. Blaszak, E. L. Ulrich, J. L. Markely and D. R. McMillin, Biochemistry **21**, 6253 (1984).
54. K. D. Olson, L. Chmurkowska-Cichowlas, C. W. McMahon and R. S. Wolfe, J. Bacteriol. **174**, 1007 (1992).
55. K. D. Olson, C. W. McMahon and R. S. Wolfe, Proc. Natl. Acad. Sci. USA **88**, 4099 (1991).

Downloaded At: 12:51 15 January 2011