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Methyl-Coenzyme M Reductase: Model Studies on Pentadentate Nickel Complexes and a Hypothetical Mechanism

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Methane is ultimately produced in methanogenic bacteria by the reaction of the methyl thioether 2-(methylthio)ethanesulfonate (methyl-coenzyme M; Me-CoM) with the thiol *N*-7-mercaptoheptanoyl-*O*-phospho-L-threonine (HS-HTP). As the second product, the mixed disulfide HTP-SS-CoM is formed. The enzyme methyl-coenzyme M reductase catalyzes this transformation and contains as cofactor the nickel tetrahydrocorphinoid "factor 430" (F_{430}). The model study described here was intended to shed light on the mechanism of this last step of methanogenesis, and in particular on the role of F_{430}. A series of nickel chelates was synthesized as models for an interaction of the sulfur containing substrates with F_{430}. As a common feature, the model complexes posess a pentadentate ligand that positions the sulfur atom of a thioether at an axial coordination site of the nickel ion. For comparison, sulfur was replaced by oxygen in one case. The electrochemical investigation of the models revealed that electron transfer from sulfur to nickel can easily take place, oxidation of the thioether moieties occured at potentials ca. 500–600 mV lower compared to those of uncomplexed thioethers (such as thioanisole). Based on this result, a hypothetical mechanism for the catalysis effected by methyl-coenzyme M reductase is formulated. The central feature of this mechanism is the initiation of sulfur–sulfur bond formation between the two substrates by the transfer of one electron from the thiolate anion of HS-HTP to F_{430} [Ni(II)]. The proposed catalytic cycle is in accord with the results of earlier studies on methyl-coenzyme M reductase (substrate analogues/inhibitors, ESR studies).

INTRODUCTION

Methanogenic archaebacteria are able to react carbon dioxide with hydrogen in a stepwise manner, ultimately affording methane and water (Eq. [1]) (*1*).

$$CO_2 + 4\,H_2 \longrightarrow CH_4 + 2\,H_2O \qquad [1]$$

The gain in free energy associated with this eight-electron reduction of CO_2 amounts to ca. 130 kJ/mol (*2*). The stepwise reduction of the C_1-unit is carried out in a "bucket brigade"-like fashion; i.e., the substrate is transferred from one cofactor/enzyme to the next as the reduction proceeds (*1*). No free C_1-intermediates such as formate are observed. The last step within the overall sequence consists in the reaction of the C_1-carrier methyl-coenzyme M (Me-CoM, **1**, Eq. [2])

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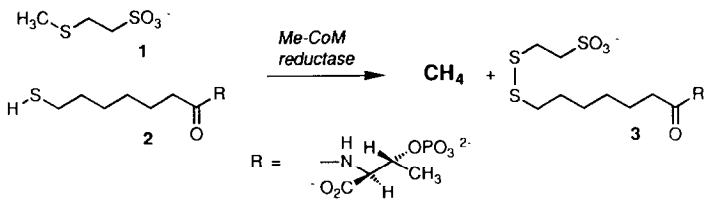
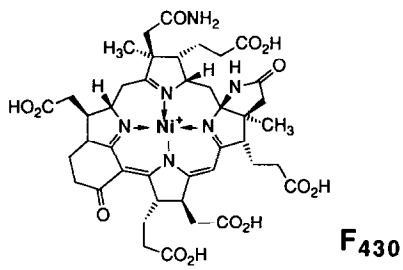


[2]

with *N*-7-mercaptoheptanoyl-*O*-phospho-L-threonine (HS-HTP, **2**, Eq. [2]). Methane and the mixed disulfide **3** (Eq. [2]) are formed as products, the reaction is catalyzed by the enzyme methyl-coenzyme M reductase (Me-CoM reductase) (*3*). The enzyme could be purified to homogeneity and is known to consist of three subunits in an $\alpha_2\beta_2\tau_2$-arrangement (*4*). Besides the two reactants **1** and **2** (Eq. [2]), the enzyme also strongly binds the nickel-containing cofactor "factor 430" (F_{430})



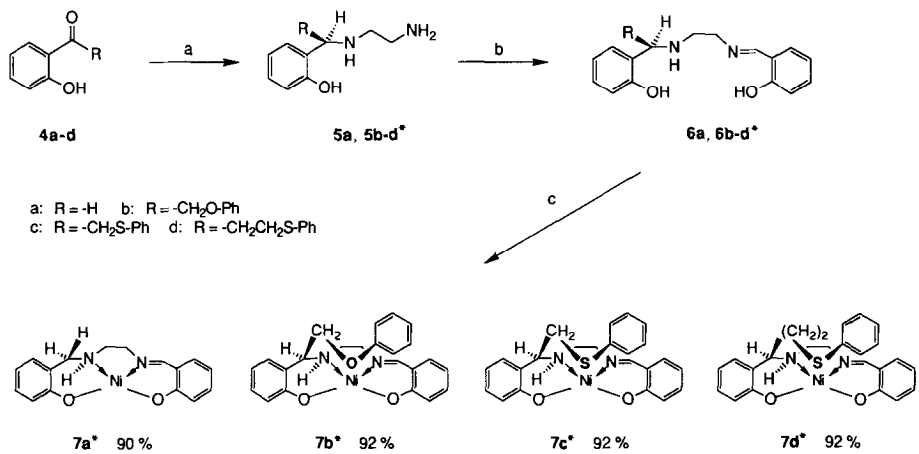
(*5*) in a stoichiometry of two F_{430} per $\alpha_2\beta_2\tau_2$ (*6*). Although no direct evidence is available so far, it appears reasonable to assume that F_{430} is involved in the catalysis effected by Me-CoM reductase. Numerous spectroscopic investigations have already dealt with the questions of (a) what the preferred conformation of the macrocyclic ligand might be like in both the bound and the free (isolated) states (*7*), (b) which mode of coordination F_{430} adopts in the enzyme (*7, 8*), and (c) whether there might be changes in the oxidation state of the nickel ion[1] during the enzymatic turnover of substrate (*9*). Based on ESR results, the involvement of F_{430} [Ni(I)] at some stage of the catalytic process appears quite likely (*9*). Furthermore, the Ni(I) state of *isolated* F_{430} (as the pentamethyl ester) could be generated and characterized (CV, UV, ESR, reactivity toward electrophiles) (*10, 11*). In the latter study it could be shown that F_{430} [Ni(I)] cleanly reacts with methyl iodide or methyl sulfonium salts to afford methane (Eq. [3]).

$$2\ F_{430}\ [Ni(I)] + CH_3\text{-}X + H^+ \longrightarrow 2\ F_{430}\ [Ni(II)] + CH_4 + X^- \qquad [3]$$

As it turned out, F_{430} [Ni(I)] did not react with thioethers, including methyl-coenzyme M (**1**, Eq. [2]).

In the light of these results, any mechanistic proposal for the catalysis effected by Me-CoM reductase must address the following questions: (a) What is the "activated form" of the thioether Me-CoM that can be demethylated by F_{430} [Ni(I)]? (b) How is the Ni(I) state of F_{430} generated during enzymatic turnover? (c)

[1] After isolation, F_{430} is in the Ni(II) state.



SCHEME 1. (a) H_2N–CH_2CH_2–NH_2, $NaBH_3CN$, (**5a**, 85%; **5b**, 76%; **5c**, 48%; **5d**, 41%); (b) salicylaldehyde, quantitative; (c) $Ni(OAc)_2 \cdot 4H_2O$. In the case of **5c**, 32% of dialkylated ethylenediamine (1 : 1 mixture of the diastereomers **8**, *rac*-**9**) were isolated as well. *Racemic mixtures.

How is the sulfur–sulfur bond between Me-CoM (**1**, Eq. [2]) and HS-HTP (**2**, Eq. [2]) established within the catalytic cycle?

Clearly, the focal point of these questions is the interaction of the nickel ion in F_{430} with the sulfur atoms of either Me-CoM (**1**) or HS-HTP (**2**). It may be assumed that in the enzyme, the sulfur atom of **1** and/or **2** occupies one of the vacant axial coordination sites of F_{430}. It therefore appeared worthwhile to synthesize model compounds that mimic such an interaction by covalently attaching a thioether moiety to a planar, four-coordinate nickel chelate. By the proper choice of the arrangement, the sulfur atom of the thioether was positioned at an axial coordination site of the nickel ion.

In this article, the synthesis and redox properties (CV) of three representives of this novel class of nickel complexes are reported. Based on the results, a mechanism for the catalysis effected by methyl-coenzyme M reductase is proposed.

RESULTS

Synthesis and Structural Characterization of the Nickel Complexes rac-7a–d

The synthesis of the complexes *rac*-**7a–d** is outlined in Scheme 1. Salicylaldehyde (**4a**) and the ketones **4b–d** were reductively aminated with ethylene diamine, affording the diamines **5a** and *rac*-**5b–d** in 41–85% yield. When the reductive amination was carried out on ketone **4c**, 32% of a 1 : 1 mixture of the diastereomeric N,N′-dialkylation products of ethylene diamine, **8** and *rac*-**9**,

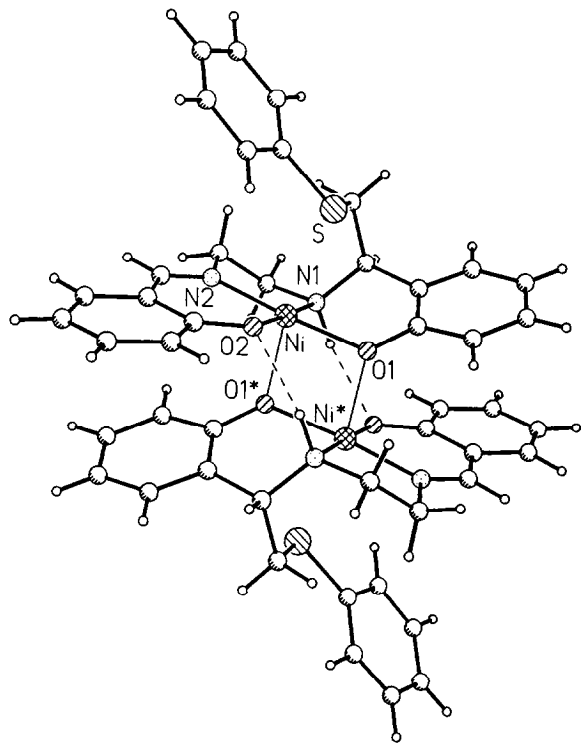


FIG. 1. X-ray structure of the nickel complex *rac*-**7c.** H bonds are shown as dashed lines, intermolecular O–Ni coordination is indicated by thin lines. Important bond distances [Å]: Ni–S, 2.575 (2); Ni–O1, 2.026 (4); Ni–O2, 1.981 (4); Ni–N1, 2.060 (5); Ni–N2, 1.989 (5); Ni–O1*, 2.109 (4).

were isolated as well. One of the diastereomers could be separated by crystallization. The ^{1}H NMR resonances of this material were doubled to two sets of equal intensity upon addition of Eu(hfc)$_3$ as chiral shift reagent. Therefore, the crystalline diastereomer is regarded as the racemic mixture of enantiomers, *rac*-**9.**

The diamines **5a** and *rac*-**5b–d** were then reacted with salicylaldehyde (**4a**) to give the *Schiff* bases **6a,** and *rac*-**6b–d.** Addition of the ligands to solutions of nickel(II) acetate resulted in the precipitation of the complexes *rac*-**7a–d.** These materials could be characterized by C,H,N analysis, IR, UV/VIS, and ^{1}H NMR spectroscopy.[2] In all cases, the analytical data were in agreement with the proposed structures. Furthermore, crystals suitable for X-ray structural analysis could be grown from complex *rac*-**7c.** A plot of the result is given in Fig. 1, together with important bond distances.[3] In the crystal, a centrosymmetrical dimer is formed by two enantiomeric molecules of the complex *rac*-**7c.** The sulfur

[2] The nickel complexes *rac*-**7a–d** turned out to be diamagnetic.

[3] See Ref (*12*) for a preliminary account of the synthesis and reactivity of complexes such as *rac*-**7c.** This reference also provides information concerning the deposition of the X-ray data set obtained from *rac*-**7c.**

TABLE 1

Cyclic Voltammetry of the Nickel Complexes *rac*-**7a–d**

Entry	Complex	$E_{1/2}$ (mV)[a]	I_c/I_a[c]
1	*rac*-**7a**	+820	0.88
2	*rac*-**7b**	+780	0.98
3	*rac*-**7c**	+750 [b]	Irreversible
4	*rac*-**7d**	+840 [b]	Irreversible
5	Thioanisole	+1350[b]	Irreversible

[a] $E_{1/2}$ vs Ag/AgCl, 3 M KCl.
[b] Potential of I_{max}.
[c] Peak separations 70–200 mV.

atom occupies an axial coordination site of the nickel ion, the octahedral coordination sphere around the metal is completed by a phenolate oxygen atom of the second (enantiomeric to the first) molecule present in the dimer.

Cyclic Voltammetry

The complexes *rac*-**7a–d** all underwent irreversible reduction at potentials around −1.4 V (vs Ag/AgCl, 3 M KCl). Obviously, this effect was independent of the presence or the structure of the sidearm.[4] The significantly differential behavior of *rac*-**7a–d** upon *oxidation* is summarized in Table 1. Both the four-coordinate chelate *rac*-**7a** and the ether complex *rac*-**7b** underwent reversible one-electron oxidation at almost identical potentials (Table 1, entries 1 and 2). The corresponding thioether complex *rac*-**7c,** however, suffered *irreversible* oxidation, again at almost the same potential (Table 1, entry 3). The same behavior was observed for the thioether complex *rac*-**7d** (Table 1, entry 4). For comparison, thioanisole (same concentration as *rac*-**7a–d**) was oxidized, too. Its irreversible oxidation wave occured at ca. +1350 mV, i.e., at a potential 500–600 mV positive of *rac*-**7c,d** (Table 1, entry 5).

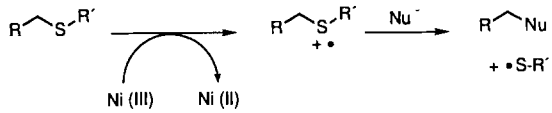
Addition of thioanisole (up to 18 mM, i.e., ca. 20-fold excess) to the solution of the four-coordinate chelate *rac*-**7a** did not affect its electrochemical behavior.

DISCUSSION

As confirmed by the X-ray crystal structure (Fig. 1), chelates such as *rac*-**7c** effectively model axial coordination of a thioether to a planar four-coordinate nickel complex. Inspection of models revealed that elongation of the side chain by one methylene group (as in *rac*-**7d**) does not adversely affect the accessibility of the axial coordination site.

[4] Irreversible reduction of nickel Schiff base complexes was observed in other cases, too. For an interpretation of this behavior, see, e.g., Ref. (*13*).

Concerning the electrochemical study, the formation of stable Ni(III) species upon anodic oxidation of Ni(II) complexes has ample precedent in the literature (*14*). In the cases of *rac*-**7a** and *rac*-**7b**, the oxidized species are stable enough to yield reversible CV wave forms. The thioether complexes *rac*-**7c,d** behave differently (Table 1, entries 3 and 4). Electron transfer from sulfur to Ni(III) can be regarded as the most reasonable explanation for their irreversible oxidation, yielding a thioether radical cation (Eq. [4]).



[4]

Such radical cations are known to be quite susceptible to the attack of nucleophiles, and may be decomposed even by the solvent acetonitrile (*15*). The observed ease of electron transfer from sulfur to nickel appears to depend on the intramolecularity of the process: The oxidation of the four-coordinate chelate *rac*-**7a** remained reversible upon addition of thioanisole. Clearly, no electron transfer to the nickel ion occurs in the case of the ether complex *rac*-**4b**.

In summary, the oxidation of a thioether axially coordinated to a nickel chelate can occur at potentials as much as 500–600 mV lower compared to the uncomplexed form (Table 1, entries 3–5). What are the possible implications of this observation for the mechanism of methyl-coenzyme M reductase?

As was pointed out in the introduction, any reasonable mechanistic proposal for the action of methyl-coenzyme M reductase must be able to explain the formation of the disulfide **3** (Eq. [2]). Sulfur–sulfur bonding generally occurs between electron-deficient species. Prominent examples are the coupling of thiyl radicals to a disulfide (Eq. [5]) (*16*), the reaction of a thiyl radical with a thioether to a sulfuranyl radical (Eq. [6]) (*17*), or the addition of a thioether radical cation to, e.g., a second thioether molecule, affording a three-electron, two-center bonded radical cation (Eq. [7]) (*18*).

[5]

[6]

[7]

Consequently, the formation of the S–S bond between methyl-coenzyme M (**1**, Eq. [2]) and HS-HTP (**2**, Eq. [2]) may be initiated by some oxidized form of either Me-CoM or HS-HTP, too. Since the Ni(I) state of F_{430} appears to play a role in the chemistry of methyl-coenzyme M reductase (*9, 11*), the *oxidation* of **1** or **2** may be coupled to the *reduction* of F_{430}. Electrochemical results on the oxidation of thioethers (*19*), thiols (*16, 20*), and thiolate anions (*16, 20*) suggest that the anion

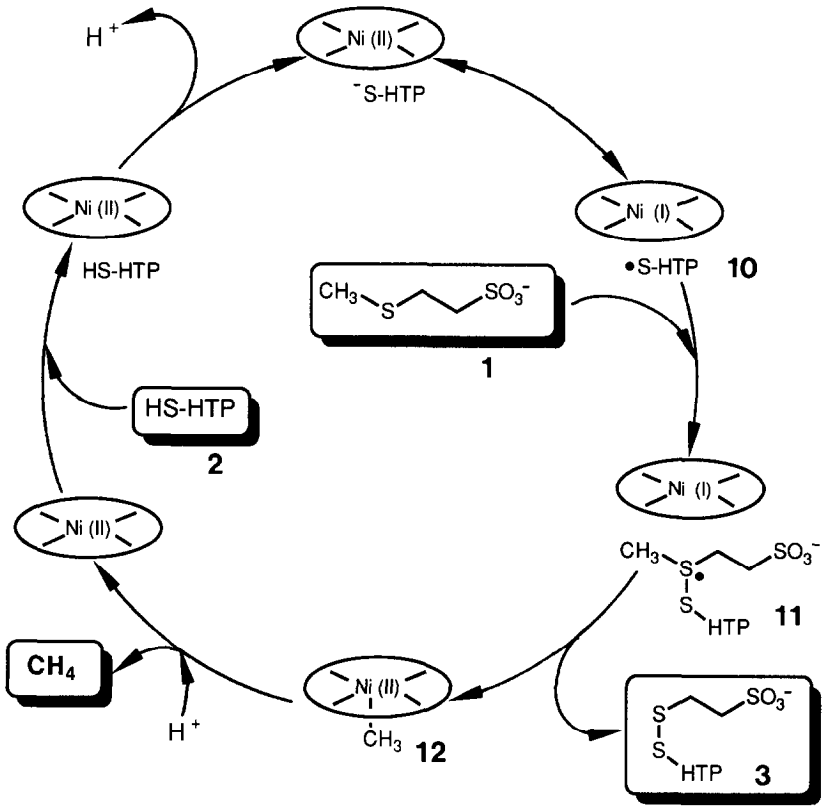


FIG. 2. Hypothetical mechanism of methyl-coenzyme M reductase.

of HS-HTP (**2**) is the most likely electron donor. In fact, thiolate anions can be oxidized at potentials as low as −500 mV (vs SCE) (*16, 20*). The reduction potential of F_{430} was found to be −840 mV (vs calomel, 0.1 M KCl)[5] (*10*). As can be judged from the model study on the complexes *rac*-**7c** and *rac*-**7d**, electron transfer from HPT-S^- to F_{430} may well be rendered thermodynamically feasible by proper orientation. A hypothetical mechanism based on this assumption is given in Fig. 2.[6]

Electron transfer from HTP—S^- to F_{430} [Ni(II)] gives rise to F_{430} [Ni(I)] and the thiyl radical **10** (Fig. 2). The latter radical can then couple with Me-CoM **1**, affording the sulfuranyl radical **11.** As mentioned before, the formation of sul-

[5] $E_{1/2}$ (vs SCE) = $E_{1/2}$ (vs calomel, 0.1 M KCl) + 92 mV.

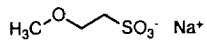
[6] It may be argued at this point that the changes in oxidation states involved in the model study on the complexes *rac*-**7a–d** [Ni(II) ⇄ Ni(III)] are not the same as those discussed for F_{430} in methyl-coenzyme M reductase [Ni(II) ⇄ Ni(I)]. However, the macrocyclic ligand of F_{430} is formally −1, whereas the ligands of *rac*-**7a–d** carry a formal charge of −2. Therefore, the overall changes take place between the uncharged species (*rac*-**7a–d** [Ni(II)], F_{430} [Ni(I)]) and those of a charge +1 (*rac*-**7a–d** [Ni(III)], F_{430} [Ni(II)]). In any case, the effect of proximity on the ease of electron transfer from sulfur to nickel is clearly demonstrated by the comparison *rac*-**7c** vs *rac*-**7a**/thioanisol.

furanyl radicals from thiyl radicals and thioethers has been observed experimentally (*17*). Demethylation of the sulfuranyl radical **11** by F_{430} [Ni(I)] affords the nickel–methyl species **12** and one of the products of the methyl-coenzyme M reductase reaction, namely, the mixed disulfide **3.** In fact, low-temperature experiments on the reductive demethylation of sulfonium salts by F_{430} [Ni(I)] (*11*) indicated the occurrence of an intermediate, most likely a nickel–methyl species. Protonation of the nickel–methyl compound **12** finally affords methane and F_{430} [Ni(II)] (Fig. 2), ready for the next catalytic cycle. The protolytic dealkylation of Ni(II)–methyl complexes has precedent in the series of simple tetraazamacrocyclic chelates (*21*) and also occurred upon addition of acid to the F_{430} [Ni(II)]–methyl intermediate mentioned above (*11*).

The crucial point of the mechanistic proposal (Fig. 2) is *the formation of the sulfuranyl radical* **11**, *initiated by oxidation at sulfur, with concomitant reduction of* F_{430} *to the Ni(I) state*. In this mechanistic scheme, the sulfuranyl radical **11** represents the "activated form" of methyl-coenzyme M (**1**).

As will be discussed now, the proposed mechanism is in agreement with the results of earlier studies on methyl-coenzyme M reductase:

(a) The oxygen-analogue of Me-CoM, 2-methoxyethanesulfonate (**13**) is an inhibitor (app. K_I = 8.3 mM) (*22*); the selena analogue (**14**) of Me-CoM is an even better substrate (higher V_{max}) than Me-CoM itself (*22*).



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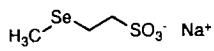

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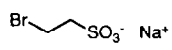
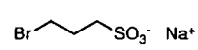
Of course, electron transfer from ether oxygen to Ni(II) (or even Ni(III), as in the electrochemical oxidation of *rac*-**4b**) does not occur. Furthermore, no precedent for a reaction of a thiyl radical with an ether (analogous to the formation of the sulfuranyl radical **11**; Fig. 2; Eq. [6]) appears to exist. On the other hand, selenoethers may even be more easily oxidized than thioethers (*23*). Therefore, the formation of a selena analogue of the sulfuranyl radical **11** (Fig. 2) appears quite feasible.

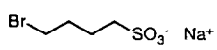
(b) ω-Bromoalkanesulfonates such as **15a–c** strongly inhibit methyl-coenzyme M reductase, 3-bromopropanesulfonate (**15b**) being the most potent inhibitor known (app. K_I = 50 nM). The inhibition is reversible (*24*).



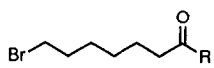
15a



15b



15c



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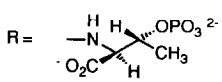
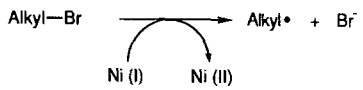


Earlier mechanistic interpretations of the methanogenesis from methyl-coenzyme M assumed a direct demethylation of the substrate by F_{430} in the Ni(I) state (*25*) and did not account for the formation of the disulfide **3** (Eq. [2]; Fig. 2). Bromoalkanes are spontaneously cleaved by Ni(I) complexes (*26*), affording bromide and an alkyl radical (Eq. [8]).



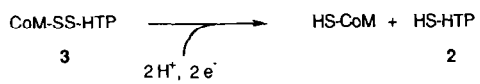
[8]

ω-Bromoalkanesulfonates as mechanistic probes should also be cleaved to an alkyl radical—if exposed to F_{430} [Ni(I)] in the enzyme. The formation of an alkyl radical within the active site should lead to irreversible damage of the enzyme (*27*). The observation of reversible inhibition is therefore in agreement with the proposed formation of F_{430} [Ni(I)] by electron transfer *from* the substrate (s).

The extremely tight binding of the bromide **15b** may be explained by its structural similarity to Me-CoM (**1**).[7] Furthermore, the bromide as a weak *Lewis* base may be coordinated to F_{430} [Ni(II)]. The latter one is known to be extremely electrophilic in the axial directions (*28*). The bromo analogue of HS-HTP, **16,** is a reversible, competitive inhibitor of methyl-coenzyme M reductase, too (app. K_I = 5 μM) (*3a*). The discussion of the inhibitory effect of **15a–c** may be applied to **16** in a similar sense.[8]

(c) Isolated Me-CoM reductase, intact cells, or cell extracts of *Methanobacterium thermoautotrophicum* show ESR signals assigned to F_{430} [Ni(I)] (*9*).

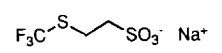
Again, this observation is in agreement with the proposed cycle, since F_{430} [Ni(I)] occurs as an intermediate. It was noted that the signals of F_{430} [Ni(I)] appeared upon incubation of intact cells with hydrogen (*9*). Reducing equivalents (such as hydrogen) are needed for the regeneration of HS-HTP (**2**) from the mixed disulfide **3** (*29*) (Eq. [9]).



[9]

In turn, HS-HTP (as the thiolate anion; Fig. 2) effects the reduction of F_{430} [Ni(II)]. Interestingly, the Ni(I) state of F_{430} persisted after exchange of hydrogen for nitrogen. This observation speaks against a direct equilibrium between the hydrogenase activity and the reduction of F_{430} [Ni(II)] and for the involvement of a mediator such as HS-HTP. Furthermore, the addition of Me-CoM (**1**) to permeabilized cells resulted in the loss of F_{430} [Ni(I)].[9] This result can be explained by the proposed mechanism, too: Introduction of Me-CoM allows for the completion of the catalytic cycle; methane and F_{430} [Ni(II)] are formed.

(d) (Trifluoromethyl)-coenzyme M (**17**) is an inhibitor (*22*).



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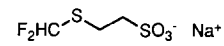
[7] In the halide **15b,** the bromine atom occupies the position of the methyl group in methyl-coenzyme M (**1**).

[8] In the bromo analogue **16,** the bromine atom occupies the position of the mercapto group of HS-HTP (**2**).

[9] Incubation with carbon dioxide resulted in the same loss of signal intensity: methyl-coenzyme M (**1**) is biosynthesized from CO_2 (*1*).

Unfortunately, no detailed studies concerning the effect of fluorination on the properties of sulfuranyl radicals like **11** (Fig. 2) appear to exist in the literature. However, a higher ionization potential was found for trifluoromethyl methyl sulfide compared to dimethyl sulfide (*30*). Consequently, the oxidation (by addition of a radical to the sulfur atom) of the former one should be less favorable. In line with this argumentation, it was concluded on the basis of preparative results that the attack of carbon-centered radicals on the sulfur atom of trifluoromethyl methyl sulfide may be less favorable compared to dimethyl sulfide (*31*). (Difluoromethyl)-coenzyme M (**18**) may be regarded as an intermediate case, since it is still reductively cleaved (*22*) to difluoromethane.



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Surely, fluorination of the methyl group does not only affect the reactivity of the thioether toward the attack of a radical. The strength of an alkyl carbon–nickel bond also is enhanced upon exchange of hydrogen for fluorine (*32*). The stabilization of the intermediate **12** (Fig. 2) may as well contribute to the inhibitory effect of (trifluoromethyl)-coenzyme M (**17**).

CONCLUSION

The nickel chelates *rac*-**7a–d** were synthesized as models for an interaction of F_{430} and the sulfur containing substrates of methyl-coenzyme M reductase. The electrochemical investigation of the complexes *rac*-**7a–d** showed that intramolecular electron transfer from sulfur to the nickel ion can easily occur. Derived from these experimental results, a hypothetical mechanism for the action of methyl-coenzyme M reductase is proposed. It is believed that the catalytic cycle is initiated by electron transfer from the thiolate anion of HS-HTP to F_{430} [Ni(II)]. A sulfuranyl radical is formulated as the "activated form" of methyl-coenzyme M which can be demethylated by F_{430} [Ni(I)]. The proposed mechanism is in accord with all other experimental evidence available so far.

EXPERIMENTAL SECTION

General

Literature procedures were used for the preparation of ketones **4b–d: 4b** (33), **4c** (34), **4d** (35). Melting points are uncorrected. Elemental analyses were carried out on a Heraeus CHN Rapid elemental analyzer. IR spectra were recorded on a Perkin–Elmer 257 instrument. ^1H NMR spectra were measured on Bruker spectrometers (250 MHz, AM 250; 270 MHz, WH 270; 300 MHz, AM 300). ^{13}C NMR spectra were taken at 75.5 MHz on a Bruker AM 300 instrument. UV/VIS spectra were recorded on a Cary 15 instrument. Cyclic voltammetry was done with

Princeton Applied Research (PAR) instrumentation: Potentiostat 173/Universal Programmer 175 or Scanning Potentiostat 362.

Syntheses

*Reductive amination of salicylaldehyde (**4a**) and of the ketones **4b–d**.* A solution of 1.80 g (30.0 mmol) ethylene diamine in 25 ml absolute methanol was placed under nitrogen and cooled with ice. Methanolic hydrochloric acid (25 mmol) was added with stirring. Methanol was added to a total volume of 35 ml and 5.00 mmol of the aldehyde/ketone was added. Stirring was continued and the temperature of the mixture was raised to ca. 20°C. When the solution had become completely homogeneous, 189 mg (3.00 mmol) sodium cyanoborohydride was added. Stirring under nitrogen at ca. 20°C was continued for 2 days. The mixture was then cooled with ice and acidified with concentrated hydrochloric acid. The solvent was removed *in vacuo* and the semisolid residue was extracted with 20 ml of a 9 : 1 (v/v) mixture of methylene chloride and methanol (the methanol was saturated with ammonia). Insoluble matter was filtered off and again extracted with 20 ml of the solvent mixture. The combined extracts were rotaevaporated and the semisolid residue was subjected to silica gel chromatography [adsorbent : substrate, 100 : 1; eluting with mixtures of methylene chloride/methanol (saturated with ammonia), eluent compositions (v/v) are stated below]. The products were freed from solvent *in vacuo* and recrystallized where possible.

4-Amino-1-(2-hycroxyphenyl)-2-azabutane (**5a**). Eluent, 9 : 1; 706 mg (85%) colorless solid which was recrystallized from ethyl acetate; colorless platelets, mp 62–63°C. Anal. Calcd for $C_9H_{14}N_2O$: C, 65.03; H, 8.49; N, 16.85. Found: C, 65.13; H, 8.46; N, 16.75. ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.68, 2.85 (mc; 4H, 3,4-H), 3.91 (br s; 3H, NH, OH), 3.98 (s; 2H, 1-H), 6.73–7.18 (m; 4H, aryl-H). IR (KBr) 3342, 3284, 3053, 2934, 2854, 1589, 1487, 1470, 1455, 1423, 1310, 1257, 1187, 1153, 1104, 1038, 939, 849, 816, 757 cm^{-1}; very broad OH/NH band at ca. 3500–2500 cm^{-1}.

(RS)-5-Amino-2-(2-hydroxyphenyl)-1-phenoxy-3-azapentane (*rac-**5b***). Eluent, 93 : 7; 1.03 g (76%) colorless oil. Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.42; H, 7.36; N, 10.22. ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.72, 2.88 (mc; 4H, 4,5-H), 4.05 (dd, J_{2H-1H} = 1.7 Hz, J_{2H-1H} = 9.3 Hz; 1H, 2–H),[10] 4.16 (J_{1H-1H} = 10.6 Hz, J_{1H-2H} = 9.3 Hz; 1H, 1-H),[10] 4.21 (J_{1H-1H} = 10.6 Hz, J_{1H-2H} = 1.7 Hz; 1H, 1-H),[10] 6.78–7.32 (m; 9H, aryl-H); very broad OH/NH at varying δ. IR (film) 3380, 3320, 3070, 3050, 2940, 2870, 1600, 1585, 1490, 1475, 1460, 1410, 1345, 1285, 1255, 1235, 1170, 1150, 1105, 1075, 1030, 1025, 910, 815, 750, 725, 685 cm^{-1}; very broad OH/NH band at ca. 3500–2500 cm^{-1}.

(RS)-5-Amino-2-(2-hydroxyphenyl)-1-(phenylthio)-3-azapentane (*rac-**5c***). Eluent, 95 : 5; 690 mg (48%) colorless oil which could be crystallized from pentane/ether at −30°C; colorless platelets, mp 63–63.5°C. Anal. Calcd for $C_{16}H_{20}N_2OS$: C, 66.63; H, 6.99; N, 9.71. Found: C, 66.82; H, 6.86; N, 9.68. ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.47–2.92 (m; 4H, 4,5-H), 3.16 (dd, J_{1H-1H} = 14.1 Hz, J_{1H-2H} = 11 Hz; 1H, 1-H), 3.32 (dd, J_{1H-1H} = 14.1 Hz, J_{1H-2H} = 3.4 Hz; 1H, 1-H), 3.69 (dd,

[10] Optimized by computer simulation (PANIC, Bruker).

J_{2H-1H} = 11 Hz, J_{2H-1H} = 3.4 Hz; 1H, 2-H), 6.71–7.58 (m; 9H, aryl-H); very broad OH/NH signals at varying δ. IR (film) 3370, 3285, 3050, 2925, 2850, 1605, 1585, 1475, 1455, 1435, 1405, 1340, 1255, 1170, 1145, 1095, 1020, 925, 820, 745, 730, 670 cm^{-1}; very broad OH/NH band at ca. 3500–2500 cm^{-1}.

Besides *rac*-**5c** (R_f 0.32), the chromatography also yielded 413 mg (32%) of a 1 : 1 mixture of the dialkylation products (2*R*, 7*S*)-2,7-bis(2-hydroxyphenyl)-1,8-bis (phenylthio)-3,6-diazaoctane (**8**) and (2*R*,7*R*/2*S*, 7*S*)-2,7-bis(2-hydroxyphenyl)-1, 8-bis(phenylthio)-3,6-diazaoctane (*rac*-**9**) as a colorless oil (R_f 0.74). Anal. Calcd for $C_{30}H_{32}N_2O_2S_2$: C, 69.74; H, 6.24; N, 5.42. Found: C, 69.72; H, 6.30; N, 5.42.

Crystallization from ethanol followed by two recrystallizations from the same solvent afforded a pure (^1H and ^{13}C NMR; constant mp) sample of one of the diastereomers: colorless platelets, mp 121°C. Anal. Calcd for $C_{30}H_{32}N_2O_2S_2$: C, 69.74; H, 6.24; N, 5.42. Found: C, 69.89; H, 6.11; N, 5.47. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.55–2.78 (m; 6H, 4,5-H, NH), 3.13 (dd, $J_{1,8H-1,8H}$ = 14.2 Hz, $J_{1,8H-2,7H}$ = 11.1 Hz; 2H, 1,8-H), 3.30 (dd, $J_{1,8H-1,8H}$ = 14.2 Hz, $J_{1,8H-2,7H}$ = 3.5 Hz; 2H, 1,8-H), 3.65 (dd, $J_{2,7H-1,8H}$ = 11.1 Hz, $J_{2,7H-1,8H}$ = 3.4 Hz; 2H, 2,7-H), 6.74–7.41 (m; 18H, aryl-H); very broad OH/NH signals at varying δ. The resonances were split to two sets of equal intensity upon addition of $Eu(hfc)_3$. ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 39.72 (t; C-4,5), 47.11 (t; C-1,8), 61.89 (d; C-2,7), 117.05 (d), 119.42 (d), 123.44 (s), 127.11 (d), 128.55 (d), 129.12 (d), 129.27 (d), 130.40 (d), 133.89 (s), 157.34 (s, aryl-C).

The ^1H and ^{13}C NMR resonances of the noncrystallizing diastereomer were determined by subtracting the spectra of the crystalline material from those of the mixture. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.55–2.78 (m; 6H, 4,5-H, NH), 3.13 (dd, $J_{1,8H-1,8H}$ = 14.2 Hz, $J_{1,8H-2,7H}$ = 10.9 Hz; 2H, 1,8-H), 3.29 (dd, $J_{1,8H-1,8H}$ = 14.2 Hz, $J_{1,8H-2,7H}$ = 3.6 Hz; 2H, 1,8-H), 3.62 (dd, $J_{2,7H-1,8H}$ = 10.9 Hz, $J_{2,7H-1,8H}$ = 3.6 Hz; 2H, 2,7-H), 6.74–7.41 (m; 18 H, aryl-H), 11.1 (br s; 2H, OH); OH/NH signals at varying δ. ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 39.69 (t; C-4,5), 46.70 (t; C-1,8), 61.70 (d; C-2,7), 117.06 (d), 119.35 (d), 123.49 (s), 127.09 (d), 128.55 (d), 129.11 (d), 129.26 (d), 130.44 (d), 133.91 (s), 157.39 (s; aryl-C).

(RS)-1-Amino-4-(2-hydroxyphenyl)-6-(phenylthio)-3-azahexane (*rac*-**5d**). Eluent, 9 : 1; 620 mg (41%) colorless oil. Anal. Calcd for $C_{17}H_{22}N_2OS$: C, 67.51; H, 7.33; N, 9.26. Found: C, 67.77; H, 7.18; N, 9.43. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.09 (mc; 2H, 5-H), 2.55–2.67, 2.72–2.95 (m; 6H, 1,2,6-H), 3.93 (dd, J_{4H-5H} = 8.2 Hz, J_{4H-5H}= 5.7 Hz; 1H, 4-H), 6.73–7.28 (m; 9H, aryl-H); OH/NH signals at varying δ. IR (film) 3302, 3055, 3004, 2929, 1608, 1586, 1480, 1456, 1438, 1411, 1353, 1258, 1188, 1176, 1151, 1096, 1071, 1025, 756, 741, 691 cm^{-1}; very broad OH/NH band at ca. 3500–2500 cm^{-1}.

*Preparation of the Nickel Complexes rac-***7a-d**. Salicylaldehyde (61 mg, 0.50 mmol) was added to a solution of 0.5 mmol of the diamines **5a** and *rac*-**5b–d** in 5 ml ethanol. The mixture turned yellow instantaneously. It was then added to a solution of 124 mg (0.50 mmol) nickel acetate tetrahydrate in 3 ml water at ca 50°C, in a dropwise manner with shaking. The beginning precipitation was driven to completion by addition of 30 ml water. The complexes were filtered off, washed with water, and dried *in vacuo* over phosphorus pentoxide. The chelates *rac*-**7a–d** were obtained as microcrystalline solids. They did not show defined melting points but decomposed upon heating.

(2-[(*RS*)-(2-](2-*Hydroxybenzylideneamino*)*ethyl*)*amino*]*methyl*]*phenolato*(2-)-*N,N',O,O'*)*nickel* (*rac*-**7a**). 147 mg (90%) orange powder. Anal. Calcd for $C_{16}H_{16}N_2O_2Ni$: C, 58.77; H, 4.93; N, 8.57. Found: C, 58.62; H, 4.84; N, 8.61. ^1H NMR (CD_2Cl_2, 250 MHz) δ 2.41–2.53 (m; 1H, HN—CH_2CH_2—N=), 2.57–2.74 (m; 1H, HN—CH_2CH_2—N=), 2.78 (dd, J_{Ar-CH_2-NH} = 12.8 Hz, J_{Ar-CH_2-NH} = 2.4 Hz; 1H, Ar-CH_2-NH), 3.11 (dd, $J_{HN-CH_2CH_2-N=}$ = 13.6 Hz, $J_{HN-CH_2CH_2-N=}$ = 5.7 Hz; 1H, HN—CH_2CH_2—N=), 3.54 (br s; 1H, NH), 3.81 (dd ps t, $J_{HN-CH_2CH_2-N=}$ = $J_{HN-CH_2CH_2-N=}$ = 13.4 Hz, $J_{NH-CH_2CH_2-N=}$ = 8.2 Hz, J = 1.6 Hz; 1H, HN—CH_2 CH_2—N=), 4.36 (ps t, J_{Ar-CH_2-NH} = J_{Ar-CH_2-NH} = 12.5 Hz; 1H, Ar-CH_2-NH), 6.20–7.15 (m; 8H, aryl-H), 6.87 (s; 1H, N=CH).[11] IR (KBr) 3450, 3260, 3030, 2920, 2860, 1630, 1600, 1535, 1480, 1450, 1345, 1330, 1305, 1280, 1265, 1205, 1145, 1125, 1075, 955, 850, 755, 745, 735 cm^{-1}. UV ($CHCl_3$) λ_{max} (log ε) 297.0 nm (3.717), 358.5 (3.622), 412.0 (3.461).

(2-[(*RS*)-*1*-[(*SR*)-(2-(2-*Hydroxybenzylideneamino*)*ethyl*)*amino*]-*2-phenoxy-ethyl*]phenolato(2-)-*N,N',O,O',O''*)*nickel* (*rac*-**7b**). 200 mg (92%) reddish brown powder. Anal. Calcd for $C_{23}H_{22}N_2O_3Ni$: C, 63.78; H, 5.12; N, 6.47. Found: C, 63.61; H, 5.32; N, 6.68. ^1H NMR (CD_2Cl_2, 270 MHz) δ 2.73 (mc; 1H, HN—CH_2CH_2—N=), 2.97–3.13 (m; 1H, HN—CH_2CH_2—N=), 3.23–3.35 (m; 2H, CH, HN—CH_2CH_2—N=), 3.70 (mc; 1H, HN—CH_2CH_2—N=), 4.59 (dd, J_{CH_2O} = 10.8 Hz, J_{CH_2O-CH} = 2.9 Hz; 1H, CH_2O), 4.80 (br ps t; 1H, NH), 6.18–7.36 (m; 13H, aryl-H), 6.40 (dd, J_{CH_2O} = 10.8 Hz, J_{CH_2O-CH} = 9.4 Hz; 1H, CH_2O), 6.65 (s; 1H, N=CH).[11] IR (KBr) 3190, 3060, 3020, 2950, 2920, 2870, 1625, 1600, 1535, 1480, 1470, 1450, 1440, 1350, 1330, 1305, 1285, 1245, 1230, 1205, 1170, 1145, 1125, 1100, 1070, 1040, 905, 875, 860, 845, 755, 745, 700, 690 cm^{-1}. UV ($CHCl_3$) λ_{max} (log ε) 298.6 nm (3.764), 358.5 (3.646), 410.4 (3.516).

(2-[(*RS*)-*1*-[(*SR*]-(2-(2-*Hydroxybenzylideneamino*)*ethyl*)*amino*]-*2*-(*phenyl-thio*)*ethyl*]*phenolato*(2-)-*N,N',O,O',S*)*nickel* (*rac*-**7c**). 207 mg (92%) brown powder. Anal. Calcd for $C_{23}H_{22}N_2O_2SNi$: C, 61.50; H, 4.94; N, 6.24. Found: C, 61.50; H, 5.19; N, 5.99. ^1H NMR ($CDCl_3$, 270 MHz) δ 2.13–2.34 (m; 1H, HN—CH_2CH_2—N=), 2.63–2.90 (m; 2H, HN—CH_2CH_2—N=, CH), 3.00–3.20 (m; 1H, HN—CH_2CH_2—N=), 4.03–4.20 (m; 1H, HN—CH_2CH_2—N=), 4.22–4.45 (m; 1H, NH), 4.51 (dd, J_{CH_2S} = 12.7 Hz, J_{CH_2S-CH} = 2.7 Hz; 1H, CH_2S), 4.80 (dd, J_{CH_2S} = 12.7 Hz, J_{CH_2S-CH} = 9.0 Hz; 1H, CH_2S), 6.03–7.45 (m; 13H, aryl-H), 6.72 (s; 1H, N=CH). IR (KBr) 3400, 3170, 3050, 3020, 2920, 2850, 1615, 1590, 1530, 1470, 1445, 1345, 1295, 1275, 1200, 1140, 1125, 1075, 1020, 950, 895, 845, 795, 745, 730, 680 cm^{-1}. UV ($CHCl_3$) λ_{max} (log ε) 298.0 nm (3.775), 355.6 (3.567), 409.0 (3.435).

(2-[(*RS*)-*1*-[(*SR*)-(2-(2-*Hydroxybenzylideneamino*)*ethyl*)*amino*]-*3*-(*phenylthio*) *propyl*]-*phenolato*(2-)-*N,N',O,O',S*)*nickel* (*rac*-**7d**). 213 mg (92%) reddish brown powder. Anal. Calcd. for $C_{24}H_{24}N_2O_2SNi$: C, 62.23; H, 5.22; N, 6.05. Found: C, 61.94; H, 5.35; N, 6.08. ^1H NMR (C_6D_6, 270 MHz) δ 1.58–1.72 (m; 1H, HN—CH_2—CH_2—N=), 1.82–2.00 (m; 1H, HN—CH_2CH_2—N=), 2.17 (dd, $J_{HN-CH_2-CH_2-N=}$ = 13.4 Hz, $J_{HN-CH_2-CH_2-N=}$ = 5.5 Hz; 1H, HN—CH_2—CH_2—N=), 2.49–2.66 (m; 2H, CH_2–CH_2S), 2.70 (br d, J = 10.9 Hz; 1H, CH), 3.07–3.19 (m; 1H,

[11] Peak assignments are based on H,H-COSY and NOE experiments.

CH_2S), 4.00 (mc; 1H, HN—CH_2—CH_2—N=), 4.12–4.26 (m; 1H, CH_2S), 4.40–4.53 (m; 1H, NH), 6.21 (s; 1H, N=CH), 6.22–7.25 (m; 13H, aryl-H). IR (KBr) 3428, 3183, 3050, 3026, 2919, 2876, 1624, 1594, 1561, 1536, 1480, 1468, 1449, 1395, 1348, 1332, 1314, 1293, 1223, 1203, 1185, 1148, 1129, 1087, 1064, 1046, 1024, 907, 870, 852, 750, 736, 692 cm^{-1}. UV ($CHCl_3$) λ_{max} (log ε) 295.0 nm (3.796), 360.4 (3.614), 412.6 (3.477).

Cyclic Voltammetry

Absolute acetonitrile was used as solvent, containing 0.1 M tetra-*n*-butylammonium perchlorate. Substrate concentrations were in the range 0.6–1.0 mM. Cyclic voltammograms were taken at a scan speed of 100 mV/s, employing platinum electrodes. In the case of *rac*-**7a**, the measurement was repeated after addition of thioanisole (up to 18 mM). The electrochemical behavior of *rac*-**7a** turned out to be unaffected by the addition of the thioether.

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