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Preliminary Communication

Methane as the product of reaction of methyl-coenzyme-M with monovalent nickel complexes in aqueous solutions. A model for the in vivo activity of cofactor F_{430}

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Abstract

Solutions containing the monovalent macrocyclic nickel complexes **1** and **2** were prepared by irradiating with ionizing radiation He saturated solutions containing the divalent complexes and 0.01 M HCO_2Na. Deaerated solutions containing methyl-coenzyme-M (MeCoM) were then injected into the vials containing the monovalent complexes. Vague traces of methane were detected at pH 7.4 while at pH 9.4 the yield of methane is over 10%. Blank experiments point out that MeCoM scavenges methyl free radicals via a mechanism which does not produce methane as the major product. A mechanism for the formation of methane in these reactions is proposed.

Keywords: Nickel complexes; Macrocyclic ligand complexes; Enzyme complexes

Nickel enzymes are known to be prominent in the metabolism of anaerobic bacteria, for example the methanogenic bacteria – the only known organisms which use CO_2 as their major electron acceptor to produce CH_4 through a series of C_1 intermediates [1].

The hydrocorphinoid nickel(II) complex, F_{430}, is proposed to be involved in the last step of methanogenesis in methanogenic bacteria as a cofactor of methyl-coenzyme-M (MeCoM) reductase, the enzyme that catalyzes the reductive cleavage of MeCoM to coenzyme-M and methane [2,3].

The macrocyclic ligand of nickel in cofactor F_{430} is a highly reduced structure described as a tetrahydrocorphin. Analysis of its conformation indicates that it can accommodate both square planar and trigonal-bipyramidal coordination geometries, a fact which is suggested to facilitate the reduction of Ni(II) to Ni(I) [4,5].

In the isolated cofactor F_{430} as well as in the resting state of the purified enzyme in aqueous solutions the nickel is present in its divalent oxidation state, Ni(II) [6,7]; however the catalytically active form of the cofactor has been proposed to involve Ni(I) [8]. This proposal is corroborated by the EPR spectra of the monovalent nickel form of MeCoM reductase [9] and of suspensions of *M. thermoautotrophicum* strain Marburg bacteria under physiological conditions [10]. Also a recent spectroelectrochemical and EPR study of factor F_{430}-Ni(II)/Ni(I) from methanogenic bacteria in aqueous solutions [11] points out that the competent form of the cofactor is presumably that in which the nickel is Ni(I).

Methane is formed when reduced cofactor F_{430}, i.e. the Ni(I) containing form, is reacted with methyl iodide or methyl sulfonium in dimethyl formamide as a solvent while the addition of MeCoM under the same conditions does not yield methane [8]. Methane is also formed when methyl chloride is catalytically reduced by Ti(III) citrate in the presence of free coenzyme F_{430} in aqueous solutions [12]. However attempts to react the reduced form of cofactor F_{430} or of model complexes with MeCoM failed to produce methane.

On the other hand MeCoM and related methyl compounds were converted to methane using the nickel macrocyclic complex [1,4,7,10,13-pentaazacyclohexadecane-14,16-dionato]nickel(II) in aqueous solutions. During this process O_2 was also formed. This process was not stimulated by reductants. Therefore it was proposed that the catalytic methane forming process from MeCoM catalyzed by this model complex involves an Ni(III)/Ni(II) couple and not an Ni(II)/Ni(I) couple as commonly accepted [13].

It seemed therefore of interest to check whether the reaction of model monovalent nickel complexes with

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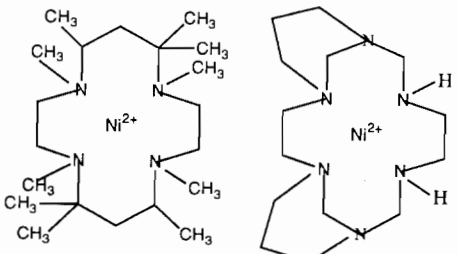
MeCoM in aqueous solutions forms methane or not. We have chosen for this study complexes **1** and **2** as they have relatively low redox potentials for the



1 **2**

couple Ni(II)/Ni(I) in aqueous solutions, −0.98 V versus SCE [14] and −1.25 V versus SCE [15] for **1** and **2**, respectively. These redox potentials are similar to that of the Ni(II)/Ni(I) couple in F_{430}, the native cofactor, −0.89 V versus SCE [11].

Complexes **1** and **2** were synthesized according to literature procedures [16,17]. MeCoM was synthesized according to the procedure reported by Taylor and Wolfe [18]:

$$BrCH_2CH_2SO_3Na + CH_3SNa + NH_4Br \xrightarrow{Ar_{sat}} CH_3SCH_2CH_2SO_3NH_4 + NaBr$$

The Ni(I) complexes were produced radiolytically as previously described [14,15]. A Co^{60} γ source or 5 MeV electrons from the linear electron accelerator of the Hebrew University of Jerusalem served as the radiation source. He saturated solutions containing 1×10^{-4} M of the divalent complexes and 0.05 M HCO_2Na at the required pH were irradiated in glass syringes with three-way valves. Under these conditions the following sequence of reactions occurs [14,15]:

$$H_2O \xrightarrow{e,\gamma} e^-_{aq}(2.65);\ H^{\cdot}(0.60);\ {}^{\cdot}OH(2.65);\ H_2(0.45);\ H_2O_2(0.75) \quad (1)$$

(where the values in parentheses give the relative yields of the primary products)

$$HCOO^- + H^{\cdot}/{}^{\cdot}OH \xrightarrow{He_{sat}} {}^{\cdot}COO^- + H_2/H_2O \quad (2)$$

$k_2(H/OH) = 2.5/2.9\times10^8/10^9\ M^{-1}\ s^{-1}$ [19]

$$Ni(II)L^i_{aq} + e^-_{aq} \xrightarrow{He_{sat}} Ni(I)L^i_{aq} \quad (3)$$

$k_3(L^1) = 8.7\times10^{10}\ M^{-1}\ s^{-1}$ [14]

$k_3(L^2) \sim 2\times10^{10}\ M^{-1}\ s^{-1}$ [15]

$$Ni(II)L^i_{aq} + {}^{\cdot}COO^- \xrightarrow{He_{sat}} Ni(I)L^i_{aq} + CO_2 \quad (4)$$

$k_4(L^1) = 4\times10^6\ M^{-1}\ s^{-1}$ [14]

$k_4(L^2) \sim 1\times10^9\ M^{-1}\ s^{-1}$ [15]

The monovalent nickel complexes thus formed are relatively long lived ($t_{1/2}$ = many hours [14] and 35 min [15] for **1** and **2**, respectively) and have distinct absorption bands in the near-UV, $\lambda_{max} = 335$ ($\epsilon = 2200\ M^{-1}\ cm^{-1}$) [14] and 374 ($\epsilon = 5560\ M^{-1}\ cm^{-1}$) [15] nm, for **1** and **2**, respectively. The concentration of the monovalent nickel complexes prepared radiolytically can thus be easily determined spectrophotometrically.

Aliquots of deaerated MeCoM ($0.1–1\times10^{-2}$ M) at pH 7.4 and 9.4 were injected into helium-saturated solutions of $Ni(I)L^i_{aq}$ ($1–2\times10^{-5}$ M) in glass vials sealed with rubber septums. GC analysis was performed a day later.

Experiments to add the MeCoM to the solutions prior to irradiation failed as under the experimental conditions some of the free radicals e^-_{aq} and ${}^{\cdot}COO^-$ reacted directly with MeCoM, reactions which lead to the formation of methane as demonstrated in blank experiments containing no nickel complexes.

No methane is detected when MeCoM is injected into vials containing non-irradiated solutions or irradiated solutions containing no nickel complexes. Vague traces of methane were detected at pH 7.4, less than 2%, while at pH 9.4 over 10% methane was detected. The yield of methane was calculated assuming that two monovalent nickel complexes are required for the production of a methane molecule. This assumption is justified independent of the detailed mechanism of methane formation which might schematically follow one of the following routes.

I. $$Ni(I)L^i_{aq} + CH_3SCH_2CH_2SO_3^- \longrightarrow Ni(II)L^i_{aq} + {}^{\cdot}CH_3 + {}^-SCH_2CH_2SO_3^-$$

followed by:

$$Ni(I)L^i_{aq} + {}^{\cdot}CH_3 \longrightarrow L^iNi(II)\text{–}CH_{3\,aq}$$

$$L^iNi(II)\text{–}CH_{3\,aq} \xrightarrow{H_2O} Ni(II)L^i_{aq} + CH_4 + OH^-$$

or by:

$$Ni(II)L^i_{aq} + {}^{\cdot}CH_3 \longrightarrow L^iNi(III)\text{–}CH_{3\,aq}$$

$$L^iNi(III)\text{–}CH_{3\,aq} \xrightarrow{H_2O} Ni(III)L^i_{aq} + CH_4 + OH^-$$

Analogous reactions were observed for the reactions of monovalent [20] and divalent [21] [1] nickel complexes, though with less crowded ligands, with alkyl radicals.

II. $$Ni(I)L^i_{aq} + CH_3SCH_2CH_2SO_3^- \xrightarrow{H^+} Ni(II)L^i_{aq} + CH_4 + {}^{\cdot}SCH_2CH_2SO_3^-$$

followed by:

$$Ni(I)L^i_{aq} + {}^{\cdot}SCH_2CH_2SO_3^- \xrightarrow{H^+} Ni(II)L^i_{aq} + HSCH_2CH_2SO_3^-$$

[1] Though heterolysis of the Ni–C bond was not observed in this study it should occur when the concentration of the transient is low as in the enzymatic system.

or by:

$$2^{\cdot}SCH_2CH_2SO_3^- \longrightarrow {}^-{}_3OS(CH_2)_2SS(CH_2)_2SO_3^-$$

followed by:

$$Ni(I)L^i_{aq} + {}^-{}_3OS(CH_2)_2SS(CH_2)_2SO_3^- \longrightarrow$$

$$Ni(II)L^i_{aq} + HSCH_2CH_2SO_3^- + {}^{\cdot}SCH_2CH_2SO_3^-$$

Reactions analogous to the latter reaction were recently reported [22].

Blank experiments in which methyl free radicals were formed in N_2O-saturated solutions containing 0.1–0.3 M $(CH_3)_2SO$ and $0–4\times10^{-3}$ M MeCoM point out that MeCoM competes with $(CH_3)_2SO$ for the methyl free radicals. Furthermore methane is not the major product of this reaction. This finding explains why the observed yield of methane is small and probably the fact that methane was not observed as a product in analogous model reactions.

Thus the results are in accord with the proposal that the mechanism of methane formation follows route I. The process leading to the formation of methane according to this scheme involves the reaction of methyl radicals with the monovalent or divalent complexes to form $L^iNi(II)–CH_3$ or $L^iNi(III)–CH_3$. This reaction is probably slow for the complexes under study due to the bulkiness of the ligands. It is even reasonable to suggest that only some of the isomers of the monovalent complexes [15] take part in this reaction. Therefore probably most of the methyl radicals react with MeCoM, the products of the latter reaction are unknown, but methane is not the major product.

Furthermore the relatively small yields of methane obtained might be partially explained by some dioxygen leakage, though maximal efforts to inhibit such leakage were made during the injection of the MeCoM-containing solutions into the vials containing the Ni(I) complexes. Both monovalent nickel complexes are known to have high rates of reaction with dioxygen [14,15].

The higher yields obtained at more alkaline pH are in accord with the recent work of Holliger et al. [11] on the redox properties of the Ni(II)/Ni(I) couple in F_{430}.

Further product analysis, detailed kinetic and mechanistic studies, and improvement of the reaction technique regarding its sensitivity to O_2 leakage are in progress.

The major conclusion of this study is that reduction of MeCoM by monovalent nickel complexes which are model compounds to cofactor F_{430} yields methane. In the enzymatic process clearly the involvement of only one nickel center can be envisaged. Thus there is no need to assume that trivalent nickel is involved in the biological process as has been proposed from previous model studies [13]. This report is, to our knowledge, the first report on the formation of methane via the reduction of MeCoM by monovalent nickel complexes with macrocyclic ligands in vitro.

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