Coenzyme F430 from Methanogenic Bacteria: Methane Formation by Reductive Carbon–Sulphur Bond Cleavage of Methyl Sulphonium Ions Catalysed by F430 Pentamethyl Ester

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The nickel(I)-form of coenzyme F430 pentamethyl ester is an efficient catalyst for the reductive cleavage of methyl sulphonium salts to methane and thioethers.

The hydrocorphinoid nickel complex coenzyme F430 (**1**)[1] is a cofactor of methyl coenzyme M reductase[2] which catalyses the reduction of methyl coenzyme M (**3**) to methane and coenzyme M (**4**)[3] in methanogenic bacteria (equation 1).

$$\underset{(\mathbf{3})}{MeSCH_2CH_2SO_3^-} \xrightarrow[\text{methyl CoM reductase (coenzyme F430) (HS-HTP)}]{2e^-,\ 2H^+} CH_4 + \underset{(\mathbf{4})}{HSCH_2CH_2SO_3^-} \quad (1)$$

Although this enzyme has been purified to homogeneity and has been investigated in several laboratories,[2] very little is known about the mechanism of the enzymic process and the specific role played by the two cofactors *N*-(7-mercaptoheptanoyl)-*O*-phosphothreonine (HS-HTP)[4] and coenzyme F430.

We recently reported that the pentamethyl ester F430M (**2**) can be reduced to the corresponding Ni^I complex without affecting the porphinoid ligand.[5] Considering the Ni^I form of coenzyme F430 as an attractive candidate for the catalytically active species which promotes carbon–sulphur bond cleavage in the enzyme, we have investigated the reactivity of Ni^IF430M towards compounds containing a methyl group bound to halogen, oxygen, or sulphur.

Ni^IF430M,[5] prepared by reduction of Ni^{II}F430M with liquid zinc amalgam in degassed dimethylformamide (DMF), reacted with iodomethane (0.5 mol. equiv.) at −60 °C within mixing time. After warming to room temperature, methane (80% yield based on MeI), Ni^{II}F430M (95%), and iodide were identified as products. Methyl tosylate at 25 °C was also converted into methane and Ni^{II}F430M, but at a much lower rate than methyl iodide. Thioethers, *e.g.* the natural substrate (**3**) or dimethyl sulphide, gave no measurable reaction under these conditions.

However, the more electrophilic methyl–sulphur bond of trimethyl sulphonium hexafluorophosphate (**5**) was cleaved by Ni^IF430M as shown in Scheme 1.

$$R^1R^2SMe^+ + 2Ni^IF430M + HX \rightarrow CH_4 + 2Ni^{II}F430M^+ + R_1R_2S + X^-$$

(**5**) $R^1 = R^2 = -Me$
(**6**) $R^1, R^2 = -(CH_2)_4-$

Scheme 1

In the absence of F430M, stirring of the sulphonium salt (**5**) with zinc amalgam for several hours produced only trace amounts of methane (see Figure 1, curve B). Therefore, it was possible to run the reaction using a catalytic amount of F430M in the presence of excess of zinc amalgam. In a typical run, F430M (1 μmol) was stirred with zinc amalgam (160 μl, 1.5% Zn) and sulphonium salt (**5**) (110 μmol) in 1 ml of degassed DMF at 25 °C. During the reaction, a steady state with more than 95% of the F430M in the Ni^I valence state was established (u.v.–vis.). After 60 h, 105 μmol (95%) of methane were found in the headspace and bulb-to-bulb distillation of the volatile components from the reaction mixture gave 95 μmol (85%) of dimethyl sulphide in the condensate. The high yields indicate a clean reaction with more than 100 turnovers of the catalyst.

Under the same conditions, the cyclic sulphonium salt (**6**) was converted into methane (70% yield), tetrahydrothiophene (67%), and methyl butyl sulphide (30%). This product ratio corresponds to a 5:1 preference for cleavage of the S–methyl bond over that of an S–alkyl bond in the five membered ring.

The kinetics of F430M-catalysed methane formation from both sulphonium ions (**5**) and (**6**) show a reproducible time lag of *ca.* 1 h in neat DMF (Figure 1, curve A). In the presence of an excess (>100 equiv. based on F430M) of n-propanethiol however, no such lag is observed and methane formation follows first order kinetics from the beginning (Figure 1, curve C). We presume that some species which has an accelerating effect similar to that of the thiol is slowly formed during the reaction in neat DMF. Hydroxide (produced from residual water) or dimethylamine formed from DMF are likely candidates. Addition of both lithium hydroxide or triethylamine did, in fact, eliminate the lag period entirely.† If the reaction was carried out in neat perdeuteriated DMF, no deuterium was incorporated into the methane produced, pointing to residual water as the proton source. With a large excess (1000-fold based on F430M) of $MeCH_2CH_2SD$ or Et_3NDCl, more than 88% of the resulting methane was CH_3D (by g.l.c.–m.s.). Since DMF is estimated to be a better donor of hydrogen atoms than both water and the triethylammonium ion,[6] these results indicate that the final step in methane formation is a proton transfer rather than a hydrogen atom abstraction. This is consistent with a mechanism involving a methyl–nickel intermediate which is then protonated to give methane and Ni^{II}F430M.‡

The rate of methane formation was found to be linearly dependent on the concentration of both F430M and the sulphonium ion. Clearly, attack of Ni^IF430M on the sulphonium ion is the rate determining step. Therefore, any F430M-derived intermediates do not accumulate. With iodomethane, which reacts much faster, the situation should be more favourable for the observation of a possible intermediate. Labelling studies indeed did provide indirect evidence for an intermediate species containing a metal-bound methyl group, although as yet we have not been able to characterize it spectroscopically. Upon mixing of Ni^IF430M and iodo-

† The origin of the accelerating effect of these additives is, at present, unclear. A possible mechanism could involve complexation to the nickel centre, thereby enhancing its reactivity towards electrophiles.

‡ Intermediate formation of an alkyl–nickel species has been postulated for the reaction of electrogenerated Ni^I-tetra-aza macrocylic complexes with n-alkyl halides.[7]

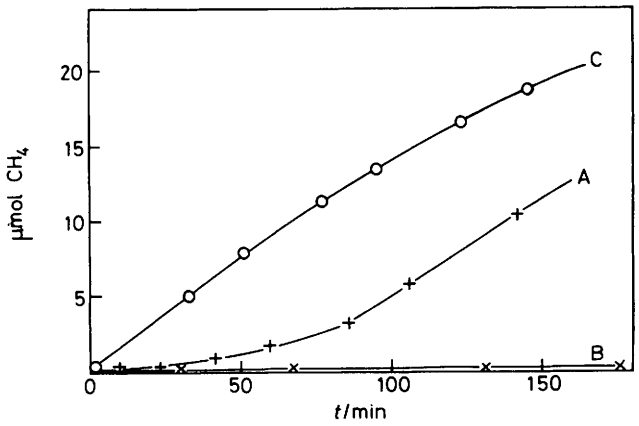

Figure 1. Kinetics of F430M-catalysed methane formation from sulphonium salt (**6**). Conditions: (**6**), (60 μmol), Zn(1.5%)–Hg (160 μl), DMF (1 ml). *T* 25 °C. (A) F430M (1 μmol); (B) n-propanethiol (160 μmol); (C) F430M (1 μmol) n-propanethiol (160 μmol).

methane in DMF at −60 °C, the colour of the solution changes instantaneously from green (Ni^{I}) to yellow. However, very little methane could be detected at that point. After warming to room temperature, methane was evolved gradually within a few minutes (85% yield, based on MeI, after 30 min). When the reaction mixture was quenched with aqueous HCl (0.1 M) immediately after the colour change, methane formation was found to be complete within seconds. If DCl–D_2O was used, the resulting methane consisted of >85% of CH_3D (g.l.c.–m.s.). Reaction with CD_3I and quenching with HCl–H_2O gave >92% CHD_3. These results give support to a mechanism which proceeds *via* a methyl–$nickel^{III}$-intermediate,§ whereas methane formation from a methyl radical which abstracts a hydrogen atom from the medium can be excluded.

§ In order to rule out the possibility that the methyl group could be intermediately bound to zinc or mercury (remaining in solution from the preparation of Ni^{I}F430M) and not to the nickel centre itself, we repeated the experiment using Ni^{I}F430M, electrogenerated at platinum electrodes. Although bulk electrolysis always led to partial decomposition of F430M and therefore to lower yields of methane, the incorporation of deuterium was in excess of 65%.

Irrespective of mechanistic questions, which will be addressed in further studies, our results demonstrate that F430M is an efficient catalyst for the reductive cleavage of carbon–sulphur bonds in methyl sulphonium ions. The close analogy to reaction (1) suggests a similar role for coenzyme F430 in the enzyme-catalysed cleavage of methyl coenzyme M to methane. The lack of reactivity of Ni^{I}F430M towards methyl CoM, however, raises the question of whether the natural substrate is converted into an activated form by the enzyme before it is reductively cleaved. On the other hand, the observed rate accelerating effect of hydroxide, amines, and thiols points to potential ways of enhancing the reactivity of the Ni^{I}-catalyst, such that methane formation from the non-activated methyl coenzyme M may become possible.

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