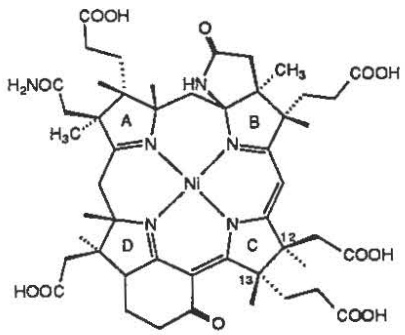
The Electronic and Magnetic Properties of Methyl-CoM Reductase and Its Nickel Cofactor F_{430}

Cristi L. Hamilton Final Seminar October 5, 1989

Methanogens are a class of strictly anaerobic archaebacteria, many of which are capable of living autotrophically on hydrogen and carbon dioxide [1]. These bacteria derive energy from the stepwise reduction of carbon dioxide to methane, a process in which the C_1 fragment is shuttled at different levels of reduction between a series of unusual cofactors [2,3]. Methyl-CoM reductase is the terminal enzyme in this pathway, and it is able to catalyze the reductive cleavage of 2-(methylthio)ethanesulfonic acid to generate methane [4]. The methyl-CoM reductase of *Methanobacterium thermoautotrophicum* (strain ΔH) is a large complex enzyme of molecular weight ~300,000 and an $\alpha_2\beta_2\gamma_2$ subunit structure [5-7]. The isolated enzyme contains two molecules of an extractable nickel(II) tetrapyrrole cofactor F_{430} that are tightly associated with the α subunits [4,8,9]. The spectroscopically determined structure of F_{430} is shown below [10,11]:



Structure of F_{430}

Variable-temperature magnetic circular dichroism (MCD) spectroscopy has been used to characterize the magnetic and electronic properties of F_{430} [12]. 4-coordinate forms are found to be diamagnetic (S = 0), whereas 6-coordinate forms are paramagnetic (S = 1). MCD studies, together with parallel low-temperature uv/visible absorption and resonance Raman investigations, show that the equilibrium distribution of 4-coordinate square-planar and 6-coordinate bis-aquo forms of the native isomer of F_{430} in aqueous solution is affected by both temperature and the presence of glycerol. Low-temperature MCD magnetization data allow the determination of the axial zero-field splitting parameter, D, of the S = 1 ground state of bis-ligand complexes of F_{430}. The value of D is sensitive to the nature of the Ni(II) axial ligands: bis-aquo F_{430}, D = +9 cm^{-1}; bis-methanol F_{430}, D = +11 cm^{-1}; bis-imidazole F_{430}, D = -6 cm^{-1}. Mea-surement of D = +9 cm^{-1} for F_{430} in the methyl-CoM reductase holoenzyme argues strongly against histidine coordination to Ni(II) in the enzyme. The possible existence of alcoholic or phenolic oxygen-containing ligands (serine, threo-nine, tyrosine, water) to Ni(II) in the enzyme-bound cofactor will be dis-cussed.

A Ni(I) form of F_{430} is believed to be important in the catalytic mechanism of methyl-CoM reductase. In order to characterize Ni(I)F_{430} chemically and spectroscopically, it is necessary to solubilize F_{430} in aprotic solvents which allow access to the negative potentials required to

generate Ni(I). These solubility properties have been achieved by amidating the five peripheral carboxylates on the F_{430} macrocycle with alkylamines by using a carbodiimide coupling method. Preliminary work in generating Ni(I) forms of these amides will be described.

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