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LETTER

Electrochemistry of Heat-extracted Methanogenic Bacterial Cofactor F₄₃₀

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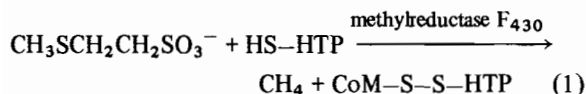
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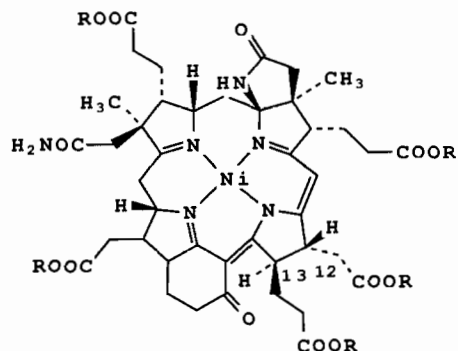
Methanogenic bacteria are the only known organisms which use CO₂ as their major electron acceptor to produce CH₄ through a series of bound C₁ intermediates [1, 2]. Based on current understanding of the last step of the overall carbon reduction sequence, the enzyme methyl coenzyme M methylreductase catalyzes the reaction of methyl coenzyme M (CH₃SCH₂CH₂SO₃⁻) and *N*-7-mercaptoheptanoyl-*o*-phosphothreonine (HS-HTP) to produce CH₄ and the heterodisulfide (CoM-S-S-HTP) [3, 4].



The methylreductase enzyme has been purified [5–8] and shown to contain 2 moles of the nickel tetrapyrrole (corphinoid) cofactor F₄₃₀, the complete structure of which has been determined [9]. Different isolation methods are known to produce different isomerized forms of F₄₃₀ [10–12]. In particular, heat-extraction procedures cause the double epimerization at both the C(12) and C(13) chiral centers in ring C of the macrocycle, resulting in the thermodynamically more stable 12,13-diepimer form of F₄₃₀, shown below [10].

Since reaction (1) is a redox reaction, there is interest in determining the electron-transfer capabilities of the nickel-containing cofactor F₄₃₀. We report here the quasi-reversible electrochemical reduction of the heat-extracted free acid form of F₄₃₀ in dimethylformamide.

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Experimental

Cultures of *Methanobacterium thermoautotrophicum*, strain YT (American Type Culture Collection No. 29183, Rockville, MD) were grown anaerobically at 60 °C under an atmosphere of 80% H₂/20% CO₂ following the procedure of Schönheit *et al.* [13], using the same growth media but with a slightly increased NiCl₂ concentration of 5 μM. Protein-free F₄₃₀ was extracted from freshly harvested or frozen cells using the heat-extraction procedure of Diekert *et al.* [14]. The F₄₃₀ was further purified by the use of a QAE-Sephadex A-25 column equilibrated with 50 mM Tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.5, and eluted with 2.5 mM HCl. The F₄₃₀ was then desalted, concentrated by ultrafiltration and lyophilized. The UV-Vis spectrum of this heat-extracted form of F₄₃₀ has a narrow peak at 430 nm, a second absorbance maximum at 273 nm, and two small shoulders at ~345 nm and ~295 nm, which are characteristic of heat-extracted F₄₃₀ and consistent with literature reports [10–12].

Cyclic voltammetry, including scan-rate studies, was carried out using a Princeton Applied Research Inc. (PAR) Model 173 potentiostat/galvanostat with a PAR Model 179 digital coulometer and PAR Model 175 universal programmer with an IBM X-Y Recorder. Solutions for electrochemical measurements were prepared in *N,N'*-dimethylformamide (dried over 4 Å molecular sieves) with tetraethylammonium perchlorate (TEAP, Southwestern Analytical Chemicals Inc., dried under vacuum at 65 °C, stored desiccated over P₂O₅) as the supporting electrolyte. Each aliquot of 5 × 10⁻⁴ M F₄₃₀ in 0.1 M TEAP/DMF was freshly prepared for each set of experiments. Potentials are relative to a BAS Ag/AgCl reference electrode which, together with the Pt wire auxiliary electrode, was isolated from the working compartment of the cell using short salt bridges. The Pt button (BAS) working electrode was freshly polished three times with 0.05 μm

alumina (BAS) and sonicated in distilled H₂O before each CV scan. All solutions were purged with N₂ for 15 min prior to each scan and a N₂ blanket was maintained over the solution.

Results

Quasireversible electrochemical behavior was observed with several heat-extracted F₄₃₀ preparations at a freshly polished Pt button electrode in 0.1 M TEAP/DMF. (At glassy carbon and gold button electrodes, the electrochemistry of F₄₃₀ was marginal, at best, in 0.1 M TEAP/DMF.) A cyclic voltammogram of heat-extracted F₄₃₀ at 20 mV/s at a Pt button electrode is shown in Fig. 1. A well-shaped voltammogram was observed with $E_{pc} = -0.84$ V, $E_{pa} = -0.73$ V and $E_{1/2} = -0.78$ V (Ag/AgCl). The peak to peak separation (ΔE_p) was 110 mV. The couple in this cyclic voltammogram is assigned to a Ni(II/I) redox process based on the equivalent current observed for both the cathodic and anodic peaks and a comparison of the observed $E_{1/2}$ value with the value reported for the non-epimerized pentamethyl ester derivative of F₄₃₀ [15–17], where the assignment to a Ni-centered redox process was confirmed by EPR and UV spectroscopy [16]. In addition, ligand hydrogenation as a result of reduction is not expected to occur in aprotic organic solvents such as DMF [16].

The diffusion coefficient, D_o , and heterogeneous rate constant, k_s , for heat-extracted F₄₃₀ were calculated from a scan-rate dependence study. The diffusion coefficient D_o can be calculated from the slope of a plot of peak current, i_p , versus (scan rate)^{1/2} using the Randles–Sevcik equation [18]. For heat-extracted F₄₃₀, the slope of the cathodic portion of the plot was $5.6 \pm 0.7 \times 10^{-6} \text{ C}^{3/2} / \text{J}^{1/2} \text{ s}^{1/2}$ ($r = 0.97$) while the anodic peak current portion had a slope of $6.2 \pm 0.1 \times 10^{-6} \text{ C}^{3/2} / \text{J}^{1/2} \text{ s}^{1/2}$ ($r = 0.99$). The average D_o value was calculated to be $4.0 \pm 0.4 \times 10^{-6} \text{ cm}^2/\text{s}$. An average heterogeneous rate constant, k_s , was also calculated from the ΔE_p data of the scan-rate studies using the method of Nicholson [19] and determined to be $6 \pm 2 \times 10^{-4} \text{ cm/s}$.

Discussion

It has been reported that heat extraction produces the isomerized 12,13-diepimer of F₄₃₀ [10–12]. Recent X-ray absorption spectroscopy (XAS) and Ni extended X-ray absorption fine structure (EXAFS) studies [11, 12] have indicated that the nickel environment found in native F₄₃₀ and the 12,13-diepimer of F₄₃₀ is different. Native F₄₃₀ is six-coordinate with rather long Ni–ligand bonds (2.10 Å) with

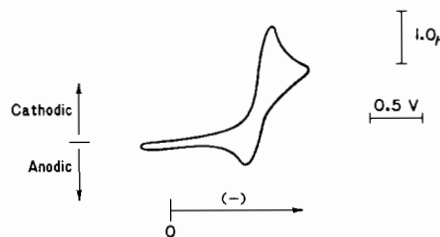


Fig. 1. Cyclic voltammogram of 5×10^{-4} M F₄₃₀ (heat-extracted) in 0.1 M TEAP/DMF. Freshly polished Pt button electrode ($A = 2.01 \times 10^{-2} \text{ cm}^2$); sweep width from +0.25 V to -1.2 V (Ag/AgCl) at 20 mV/s.

tetragonally distorted octahedral geometry and a nearly planar macrocycle ring [11, 12]. In contrast, the 12,13-diepimer of F₄₃₀ is four-coordinate with short Ni–ligand bonds (1.89 Å), square-planar geometry, and most likely a ruffled macrocycle core [11, 12]. However, comparison of our results for the redox potential of the heat-extracted free acid 12,13-diepimer of F₄₃₀ ($E_{1/2} = -0.78$ V versus Ag/AgCl) with that of the non-heat-extracted pentamethyl ester derivative of F₄₃₀ ($E_{1/2} = -0.86$ V versus SCE [17]) suggests that corphinoid ring epimerization and/or ester derivatization have little influence on the redox potential of the F₄₃₀ cofactor.

The value of D_o for heat-extracted F₄₃₀ in 0.1 M TEAP/DMF of $4.0 \pm 0.4 \times 10^{-6} \text{ cm}^2/\text{s}$ is somewhat less than values reported for various nickel(II) tetraamine macrocyclic complexes [20], but is within the range of 10^{-6} to $10^{-5} \text{ cm}^2/\text{s}$ for a typical metallo-macrocycle diffusion coefficient [21]. However, the heterogeneous electron-transfer rate constant, k_s , for heat-extracted F₄₃₀ in 0.1 M TEAP/DMF of $6 \pm 2 \times 10^{-4} \text{ cm/s}$ is approximately two orders of magnitude less than the k_s values reported for various nickel(II) porphyrins and nickel(II) tetramethyl chlorin [22] at a Pt electrode. Since both the F₄₃₀ [16] and nickel(II) porphyrin reductions [23] cited above are nickel centered, it appears that these differences in rate constants are a function of the influence of the macrocycle ligand on the reduction of Ni(II) to Ni(I). The magnitude of k_s for heat-extracted F₄₃₀ characterizes the electron transfer at the electrode surface as quasi-reversible ($10^{-6} < k_s < 10^{-3} \text{ cm/s}$), while nickel(II) porphyrins exhibit reversible behavior ($k_s > 10^{-2} \text{ cm/s}$).

Much emphasis has been placed on the reported difference in electrophilicity between native F₄₃₀ and the 12,13-diepimer of F₄₃₀ [10–12, 15]. A comparison of the heterogeneous electron-transfer rate constant k_s for both native F₄₃₀ and the 12,13-diepimer of F₄₃₀ may provide some insight on the influence of ring epimerization on facility of electron transfer. Unfortunately, a k_s value for native F₄₃₀ has not yet been reported so this comparison cannot be made.

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