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CO and O_2 binding studies of new model complexes for C*c*O

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ABSTRACT

A very efficient, simple synthesis of a porphyrin mimic for cytochrome *c* oxidase is presented. This complex contains a covalently attached copper binding site and a phenyl ring that mimics the Tyr part of the enzyme. Structural studies revealed that the copper binding tridentate ligand is situated on top of the porphyrin plane and the hydroxyl of the phenyl ring is oriented towards the center of the molecule. Moreover, Fe at the reduced form of the model is capable of binding CO, as confirmed by FT-IR and upon photolysis CO is bound to Cu site. Finally, electrochemical studies using rotating ring-disk electrode showed that the complexes reduce oxygen via two mechanisms ($2e^-$ and $4e^-$) and have low stability.

1. Introduction

Cytochrome *c* oxidase (C*c*O) is the terminal electron acceptor of the mitochondrial electron transport chain and is responsible for the four-electron reduction of dioxygen to water [1–4]. The energy released in this reaction is used to produce a proton gradient across the inner mitochondrial membrane, which provides the driving force for the ATP synthesis [5]. X-ray crystallographic analysis revealed that the catalytic active site of the enzyme consists of a heme (heme a_3), a tricoordinated copper ion (Cu_B) and a tyrosine residue (Y244) which is covalently connected to one of the copper bound histidines (H240) (Fig. 1) [2,6–9]. The reduction of dioxygen to water takes place at the heme a_3–Cu_B binuclear center, with an iron–copper distance of about 5 Å. During the catalytic process dioxygen is bound to Cu_B, followed by transfer of O_2 to heme a_3, to form the first intermediate (Fe^{III}-superoxo). After cleavage of O–O bond, species such as ferryl-oxo (Fe^{IV}=O) and Cu_B^{II}–OH are formed. Moreover, the above O–O cleavage must be clarified. Also, a bridging peroxo species (Fe^{III}–O–O–Cu^{II}) has been proposed as a possible intermediate, but still has not been observed at the enzymatic reaction [10–14]. In addition the crosslinked Tyr is thought to participate in catalysis by providing the fourth electron needed to cleave the O–O bond in a net hydrogen abstraction [15]. Regarding all the above information, several points have not yet been clarified such as, the participation of Cu_B in the oxygen activation reaction, the Tyr role and the characterization of transient species. Therefore, the not fully understood mechanism of dioxygen reduction by C*c*O has inspired many chemists to model aspects of its active site and related chemical properties. A series of synthetic models have been developed and studied by Collman [16–20], Karlin [21–25], Naruta and others [26,27]. Following our previous efforts to understand the specific functions of cytochrome *c* oxidase [28–30], we have successfully prepared and studied a novel enzyme mimic. This model contains a copper binding site, situated above the porphyrin ring and a tyrosine mimic, covalently attached onto the porphyrin.

2. Experimental

2.1. General

^{1}H NMR spectra were recorded unless otherwise specified, as deuteriochloroform solutions using the solvent peak as internal standard on a Bruker AMX-500 MHz spectrometer. UV–Vis spectra were recorded on a Shimadzu Multispec-1501 instrument. High-resolution mass spectra were performed on a Bruker ultrafleXtreme MALDI-TOF/TOF spectrometer using trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile as a matrix. Elemental analyses were carried out using a Carlo-Erba EA 1110, CHNS Eager 200 analyzer. Thin layer chromatography was performed on silica gel 60 F_{254} plates. Chromatography refers to flash chromatography and was carried out on SiO_2 (silica gel 60, SDS, 70–230 mesh ASTM). All dry solvents used were dried by the appropriate technique. Organic extracts were dried over magnesium sulfate unless indicated otherwise. Evaporation of the solvents was accomplished

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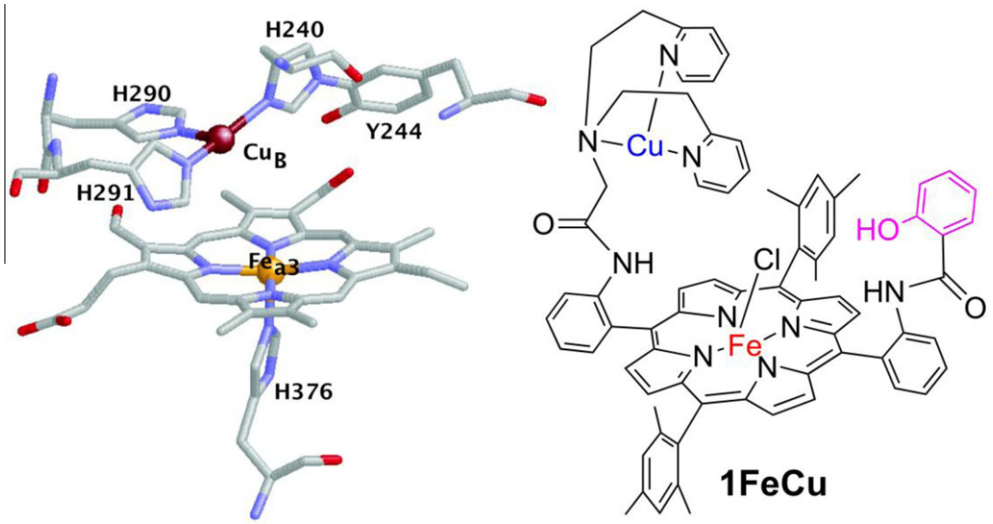

Fig. 1. (A) CcO-active center, (B) model compound **1FeCu** (the Cu counter anions are not represented).

on a rotary evaporator. Synthesis and characterization of all free base porphyrins has been reported elsewhere [20].

2.2. Synthesis of α-5-[2-(bis-(2-(pyridin-2-yl)ethyl)amino)-acetamidophenyl]-α-15-[2-((2-methoxy)benzamide) phenyl]-10,20-bis-(2,4,6-trimethyl-phenyl)-porphyrin (**3**)

2-Methoxy benzoic acid (34 mg, 0.22 mmol) and 1,3 dicyclohexylcarbodiimide (DCC) (48 mg, 0.23 mmol) were added to a solution of porphyrin **2** (80 mg, 0.08 mmol) in CH_2Cl_2 (4 mL). The resulting mixture was stirred for 48 h at 5 °C, after which time CH_2Cl_2 (20 mL) was added and washed with water (4 × 30 mL). The organic layer dried, filtered, concentrated and the residue was chromatographed on a silica gel column (0–6% ethanol in dichloromethane). Compound **3** was eluted with CH_2Cl_2/EtOH 50:3 and obtained as a purple solid (77 mg, 85%). R_f (CH_2Cl_2/MeOH 9:1): 0.50. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.89 (s, 1H, H_{32}), 9.65 (s, 1H, H_{22}), 9.06 (d, J = 8.5 Hz, 1H, H_4), 8.86 (d, J = 8 Hz, 1H, H_{18}), 8.82 (m, 4H, H_{pyr}), 8.70 (d, J = 4.5 Hz, 2H, H_{pyr}), 8.67 (d, J = 4.5 Hz, 2H, H_{pyr}), 8.05 (m, 1H, H_{35}), 7.87 (m, 4H, H_1, H_3, H_{15}, H_{17}), 7.79 (d, J = 4 Hz, 2H, H_{31}), 7.47 (m, 2H, H_2, H_{16}), 7.22 (s, 2H, H_{10}), 7.14 (s, 2H, H_{12}), 6.72 (m, 2H, H_{36}, H_{37}), 6.36 (t, J = 7 Hz, 2H, H_{30}), 6.28 (t, J = 7 Hz, 2H, H_{29}), 5.30 (m, 1H, H_{38}), 4.59 (d, J = 7 Hz, 2H, H_{28}), 2.72 (s, 2H, H_{24}), 2.59 (s, 6H, $H_{p\text{-meth}}$), 1.68 (s, 6H, $H_{o\text{-meth}}$), 1.54 (m, 4H, H_{25}), 1.44 (s, 6H, $H_{o\text{-meth}}$), 0.77 (m, 4H, H_{26}), 0.22 (s, 3H, H_{40}), −2.38 (s, 2H, H_{NHpyr}). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.6 (C_{23}), 163.4 (C_{33}), 157.6 (C_{27}), 156.2 (C_{39}), 148.3 (C_{31}), 139.8 (C_{13}), 139.5 (C_5), 138.6 (C_9), 138.5 (C_{19}), 138.2 (C_{11}), 137.6 (C_8), 135.7 (C_{29}), 135.4 (C_1), 135.2 (C_{15}), 132.8 (C_{37}), 132.1 (C_{35}), 131.7 (C_6), 131.5 (C_{20}), 129.9 (C_3), 129.8 (C_{17}), 128.1 (C_{10}), 127.9 (C_{12}), 123.0 (C_{16}), 122.6 (C_2), 122.0 (C_{28}), 121.6 (C_4), 120.9 (C_{36}), 120.8 (C_{30}), 120.7 (C_{34}), 120.5 (C_{18}), 119.2 (C_{14}), 115.1 (C_{21}), 114.5 (C_7), 110.5 (C_{38}), 58.2 (C_{24}), 54.2 (C_{25}), 52.5 (C_{40}), 34.3 (C_{26}), 21.7 ($C_{o\text{-meth}}$), 21.5 ($C_{o\text{-meth}}$), 21.3 ($C_{p\text{-meth}}$). UV–Vis (CH_2Cl_2): λ_{max} (logε, mM^{-1} cm^{-1}): 420 (335.2), 515 (16.9), 548 (4.6), 589 (4.8), 645 (2.2). *Anal.* Calc. for $C_{74}H_{67}N_9O_3$: C, 78.63; H, 5.97; N, 11.15. Found: C, 78.58; H, 5.95; N, 11.19%. HRMS (MALDI-TOF): m/z calc for $C_{74}H_{68}N_9O_3$, 1130.5445 $[M+H]^+$; found: 1130.5449.

2.3. Synthesis of α-5-[2-(bis-(2-(pyridin-2-yl)ethyl)amino)-acetamidophenyl]-α-15-[2-((2-hydroxyl)benzamide) phenyl]-10,20-bis-(2,4,6-trimethyl-phenyl)-porphyrin (**1**)

To a solution of porphyrin **3** (34 mg, 0.03 mmol) in dry CH_2Cl_2 (30 mL) a solution of BBr_3 (1.0 M, 0.35 ml, 0.35 mmol) in dry CH_2Cl_2 was added and the mixture was stirred under argon at −78 °C for 30 min and at 0 °C for 1 h. The reaction was quenched with MeOH (8 mL) and H_2O (16 mL) and stirred for 20 min at 0 °C. The mixture was washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and water (2 × 20 mL). The organic phase was dried, and the solvent was removed under reduced pressure. The crude material was purified by column chromatography on a silica gel column (0–6% methanol in dichloromethane). Compound **1** was eluted with CH_2Cl_2/MeOH 50:3 and obtained as a purple solid (31 mg, 93%). R_f (CH_2Cl_2/MeOH 9:1): 0.49. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.66 (s, 1H, H_{32}), 9.48 (s, 1H, H_{22}), 8.96 (d, J = 8.5 Hz, 1H, H_4), 8.91 (d, J = 8 Hz, 1H, H_{18}), 8.85 (d, J = 4.5 Hz, 2H, H_{pyr}), 8.80 (d, J = 5 Hz, 2H, H_{pyr}), 8.68 (d, J = 5 Hz, 2H, H_{pyr}), 8.61 (d, J = 4.5 Hz, 2H, H_{pyr}), 8.19 (dd, J_1 = 7 Hz, J_2 = 1 Hz, 1H, H_1), 8.03 (d, J = 7.5 Hz, 1H, H_{15}), 7.90 (t, J = 7.5 Hz, 1H, H_3), 7.85 (t, J = 7.5 Hz, 1H, H_{17}), 7.57 (t, J = 7.25 Hz, 1H, H_2), 7.51 (t, J = 7.5 Hz, 1H, H_{16}), 7.46 (d, J = 4 Hz, 2H, H_{31}), 7.19, (s, 2H, H_{10}), 7.12 (s, 2H, H_{12}), 6.94 (d, J = 7 Hz, 1H, H_{35}), 6.75 (t, J = 7 Hz, 2H, H_{29}), 6.57 (t, J = 6 Hz, 2H, H_{30}), 6.43 (t, J = 7.5 Hz, 1H, H_{37}), 5.97 (t, J = 7 Hz, 1H, H_{36}), 5.46 (d, J = 8 Hz, 1H, H_{38}), 5.28 (d, J = 7.5 Hz, 2H, H_{28}), 2.65 (s, 2H, H_{24}), 2.57 (s, 6H, $H_{p\text{-meth}}$), 1.60 (s, 6H, $H_{o\text{-meth}}$), 1.37 (s, 6H, $H_{o\text{-meth}}$), 1.25 (m, 4H, H_{25}), 0.52 (m, 4H, H_{26}), –2.40 (s, 2H, H_{NHpyr}). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8 (C_{23}), 165.7 (C_{33}), 157.7 (C_{39}), 157.5 (C_{27}), 148.2 (C_{31}), 140.0 (C_{13}), 139.8 (C_5), 139.1 (C_{19}), 138.9 (C_9), 138.4 (C_{11}), 137.9 (C_8), 136.5 (C_{29}), 135.3 (C_1), 134.9 (C_{15}), 133.1 (C_{37}), 132.4 (C_6), 131.4 (C_{20}), 130.2 (C_{17}), 130.1 (C_3), 129.0 (C_{35}), 128.3 (C_{10}), 128.1 (C_{12}), 123.3 (C_2), 123.1 (C_{16}), 122.5 (C_{28}), 122.0 (C_4), 121.3 (C_{30}), 120.0 (C_{18}), 119.3 (C_{36}), 119.1 (C_{14}), 117.8 (C_{34}), 116.6 (C_{38}), 114.8 (C_7), 114.3 (C_{21}), 58.9 (C_{24}), 54.0 (C_{25}), 34.0 (C_{26}), 21.9 ($C_{o\text{-meth}}$), 21.8 ($C_{o\text{-meth}}$), 21.6 ($C_{p\text{-meth}}$). UV–Vis ($CHCl_3$): λ_{max} (ε, mM^{-1} cm^{-1}): 420 (340.5), 515 (17.0), 548 (4.7), 589 (4.8), 645 (2.3). *Anal.* Calc. for $C_{73}H_{65}N_9O_3$: C, 78.54; H, 5.87; N, 11.29. Found: C, 78.51; H, 5.96; N, 11.33%. HRMS (MALDI-TOF): m/z calc for $C_{73}H_{66}N_9O_3$, 1116.5289 $[M+H]^+$; found: 1116.5293.

2.4. Synthesis of 2-methoxy-N-phenylbenzamide (**6**)

A mixture of aniline (0.24 mL, 2.6 mmol), 2-methoxy benzoic acid (0.40 g, 2.6 mmol) and 1-hydroxy-1H-benzotriazole (HOBt) (0.43 g, 3.2 mmol) in THF (10 mL), was cooled at −10 °C. A solution of 1,3 dicyclohexylcarbodiimide (DCC) (0.65 g, 3.2 mmol) in THF (3 mL) was added to the above solution and the mixture was stirred at −10 °C for 1 h. The resulting mixture was then stirred at room temperature for 12 h and filtered. The filtrate was collected