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Spectroscopic and electrochemical studies of novel model compounds for cytochrome *c* oxidase

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1. Introduction

The consumption of oxygen in aerobic cellular respiration is primarily mediated by the multiunit enzyme cytochrome *c* oxidase (CcO) [1,2]. This system spans the mitochondrial membrane and catalyzes the reduction of dioxygen to water while concomitantly building a transmembrane electrochemical gradient by proton translocation. Thus, dioxygen serves the dual role as terminal electron acceptor for oxidative catabolism and as energy source for the majority of ATP production. The three-dimensional structures of CcOs from *Paracoccus denitricans* [3,4] and bovine heart [5–7] have been determined by X-ray diffraction. 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).

Extensive spectroscopic and crystallographic studies of CcO have illuminated many characteristics that lead to its efficient 4e⁻ reduction of dioxygen, although many controversial issues remain, especially concerning the role of the copper ion and the tyrosine residue. The development of biomimetic models to investigate the structural–functional relationships of native metalloenzymes has proven to be a successful strategy [8–11]. Simulation and variation of synthetic models provide insight into the coordination environments, spectroscopic properties, and catalytic mechanisms

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ABSTRACT

Different metalated porphyrin compounds were studied as model complexes for cytochrome *c* oxidase. All models contain a tyrosine molecule and a copper binding site. Two of the compounds are bearing an axial pyridine ligand that could possibly coordinate with Fe porphyrins. All complexes were studied using NMR and UV–Vis spectroscopies and it was found that the coordination of the axial ligand is possible only in one of the porphyrins. Moreover, the synthesized catalysts were studied as promising enzyme mimics using a rotating disc electrode in the presence of molecular oxygen.

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of metalloenzymes. Such detailed information and systematic variations are difficult to obtain from wild-type enzymes or their mutants because of their restricted availability and difficulty in mutagenesis. To date, many synthetic models have been reported by Karlin and co-workers [12–15] and Collman et al. [16–19] with models bearing either a Tyr244 mimic or not. However, in all of the cases the tyrosine mimic is only a phenol ring and not the whole tyrosine molecule. In the present study, we have developed a series of models bearing a tyrosine molecule and an axial ligand that could coordinate with iron and block the lower face of the model, leaving the upper face free to bind an oxygen molecule.

2. Experimental

2.1. General

¹H 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 MS/MS ZABSpec TOF spectrometer at the University of Rennes I (C.R.M.P.O.). Elemental analyses were carried out using a Carlo-Erba EA 1110, CHNS Eager 200 analyzer. Thin layer chromatography was performed on silica gel 60 F₂₅₄ plates. Chromatography refers to flash chromatography and was carried out on SiO₂ (silica gel 60, SDS, 70–230 mesh ASTM). All dry solvents used were dried by the appropriate technique. Organic extracts were dried over





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Fig. 1. Structure of the bovine cytochrome *c* oxidase active center.

magnesium sulfate unless indicated otherwise. Evaporation of the solvents was accomplished on a rotary evaporator. Synthesis and characterization of all free base porphyrins has been reported elsewhere [20].

2.2. General procedure of Fe metalation reactions

In a Schlenk flask and under an inert atmosphere, 1 equivalent of porphyrin and 10 equivalents of iron dibromide (FeBr₂) were added. Then deoxygenated and anhydrous THF inserted in the flask and the solution was stirred at 55 °C for 12 h. The color of the solution changed from purple to orange. The completion of the reaction was confirmed by UV–Vis spectroscopy and TLC chromatography. The solvent removed under vacuum and the residue dissolved in chloroform and washed twice with 2 N HCl, then once with saturated NaHCO₃, dried, filtered, and concentrated under vacuum. The resulting product was purified by column chromatography and recrystallized in CH₂Cl₂ pentane, to obtain Fe porphyrin, as brown-purple solid. Throughout the purification procedures excess of NaCl was used in order to confirm that a Cl atom remains as the axial Fe ligand.

2.2.1. Synthesis of porphyrin 1Fe

Porphyrin **1** (30 mg, 0.026 mmol) and FeBr₂ (56 mg, 0.26 mmol) were dissolved in THF (5 ml). Column chromatography (1–5% MeOH in chloroform). The product was eluted at 2% MeOH in chloroform to give solid **1Fe** (27 mg, 82%). $R_{\rm f}$ (CH₂Cl₂/MeOH 9:1): 0.71. *Anal.* Calc. for C₇₄H₆₄N₁₀O₅FeCl: C, 70.28; H, 5.10; N, 11.08. Found: C, 70.09; H, 5.23; N, 11.13%. UV–Vis absorption: $\lambda_{\rm max}$, nm (ε , cm⁻¹, M⁻¹), 418 (9.5 × 10⁴), 509 (2.4 × 10³). HRMS (ES⁺) Calc. for C₇₄H₆₄N₁₀O₅Fe [M–Cl]⁺ 1228.4411. Found: 1228.4435.

2.2.2. Synthesis of porphyrin 2Fe

Porphyrin **2** (15 mg, 0.012 mmol) and FeBr₂ (26 mg, 0.12 mmol) were dissolved in THF (3 ml). Column chromatography (1–10% MeOH in chloroform). The product was eluted at 5% MeOH in chloroform to give solid **2Fe** (13 mg, 78%). $R_{\rm f}$ (CH₂Cl₂/MeOH 9:1): 0.41. *Anal.* Calc. for C₈₂H₇₄N₁₂O₄FeCl: C, 71.22; H, 5.39; N, 12.15. Found: C, 71.39; H, 5.22; N, 12.04%. UV–Vis absorption: $\lambda_{\rm max}$, nm (ε , cm⁻¹, M⁻¹), 418 (8.4 × 10⁴), 507 (2.1 × 10³). HRMS (ES⁺) Calc. for C₈₂H₇₄N₁₂O₄Fe [M–Cl]⁺ 1346.5305. Found: 1346.5319.

2.2.3. Synthesis of porphyrin 3Fe

Porphyrin **3** (40 mg, 0.031 mmol) and FeBr₂ (67 mg, 0.31 mmol) were dissolved in THF (7 ml). Column chromatography (1-10% MeOH in chloroform). The product was eluted at 5% MeOH in chlo-

roform to give solid **1Fe** (34 mg, 79%). R_f (CH₂Cl₂/MeOH 9:1): 0.45. Anal. Calc. for C₈₂H₇₄N₁₂O₄Fe: C, 73.10; H, 5.54; N, 12.47. Found: C, 73.01; H, 5.44; N, 12.58%. UV–Vis absorption: λ_{max} , nm (ε , cm⁻¹, M⁻¹), 415 (8.5 × 10⁴), 506 (2.3 × 10³). HRMS (ES⁺) Calc. for C₈₂H₇₄N₁₂O₄Fe [M]⁺ 1346.5305. Found: 1346.5321.

2.3. General procedure of Cu metalation reactions

To a solution of 1 equivalent of iron porphyrin in a mixture of MeCN/EtOH (95:5) 1.1 equivalents of copper acetate (dissolved in MeCN), was added and the mixture was stirred at 60 °C for 10 min. The completion of the reaction was confirmed using TLC chromatography. Then, the solvent was removed under vacuum and the resulting solid was dissolved in chloroform. The solid was precipitated using pentane, filtered and washed with pentane giving FeCu porphyrin as brown-purple solid.

2.3.1. Synthesis of porphyrin 1FeCu

Porphyrin **1Fe** (10 mg, 7.9 µmol) and copper acetate [(0.8 ml, 8.7 µmol), from a standard solution of 50 mg of Cu(CH₃COOH)₂ in 25 ml of MeCN] were dissolved in MeCN/EtOH (5 ml). Product **1FeCu** was obtained quantitatively. R_f (CH₂Cl₂/MeOH 9:1): 0.70. UV–Vis absorption: λ_{max} , nm (ϵ , cm⁻¹, M⁻¹), 418 (9.0 × 10⁴), 509 (2.2 × 10³). HRMS (ES⁺) Calc. for C₇₄H₆₄N₁₀O₅FeCu [M–Cl]⁺ 1291.3707. Found: 1291.3715.

2.3.2. Synthesis of porphyrin 2FeCu

Porphyrin **2Fe** (7 mg, 5.1 µmol) and copper acetate [(0.5 ml, 5.6 µmol), from a standard solution of 50 mg of Cu(CH₃COOH)₂ in 25 ml of MeCN] were dissolved in MeCN/EtOH (4 ml). Product **2FeCu** was obtained quantitatively. *R*_f (CH₂Cl₂/MeOH 9:1): 0.41. UV–Vis absorption: λ_{max} , nm (ϵ , cm⁻¹, M⁻¹), 417 (8.2 × 10⁴), 507 (2.0 × 10³). HRMS (ES⁺) Calc. for C₈₂H₇₄N₁₂O₄FeCu [M–Cl]⁺ 1409.4601. Found: 1409.4617.

2.3.3. Synthesis of porphyrin 3FeCu

Porphyrin **3Fe** (20 mg, 14.5 µmol) and copper acetate [(1.5 ml, 15.9 µmol), from a standard solution of 50 mg of Cu(CH₃COOH)₂ in 25 ml of MeCN] were dissolved in MeCN/EtOH (8 ml). Product **3FeCu** was obtained quantitatively. $R_{\rm f}$ (CH₂Cl₂/MeOH 9:1): 0.44. UV–Vis absorption: $\lambda_{\rm max}$, nm (ε , cm⁻¹, M⁻¹), 415 (8.0 × 10⁴), 509 (2.1 × 10³). HRMS (ES⁺) Calc. for C₈₂H₇₄N₁₂O₄FeCu [M]⁺ 1409.4601. Found: 1409.4612.

2.4. Electrochemical studies

The diameter of the graphite disk of the RRDE (Pine Instruments) was 6 mm, a bipotentiostat (Solea-Tacussel) was used to control its potential and maintained the platinum ring potential at 1.0 V so as to detect, through its oxidation, H_2O_2 produced by the reduction of O_2 at the graphite electrode. The voltammograms were recorded on a SEFRAM T.2Y *x*-*yy* recorder. All the potentials are referred to SCE (KCl saturated). The aqueous solution in contact with the electrode was buffered at pH 6.86 (0.025 M KH₂PO₃, 0.025 M Na₂HPO₃) and saturated with O_2 at 1 atm. The electrode was abraded with wet SiC paper (grade 600), sonicated in deionized water for 1 min, washed with water and acetone, and dried. The adsorption of the catalysts on the graphite surface was done by immersing the electrode was then rinsed with CH₂Cl₂ and used for the measurements.

3. Results and discussion

3.1. Chemical structures of synthesized models

The synthesis of novel free base porphyrins 1-3 (Scheme 1), was previously reported by our group [20]. The series of these complexes are featuring a covalently attached tridentate ligand site, where a Cu could be coordinated, a tyrosine molecule, and two of them contain a proximal pyridine base. These new models are easily made using the α,β atropisomer of the same porphyrin. The hydroxyl group of all compounds can be protected/deprotected in order to investigate the role of this tyrosine mimic. The tridentate ligand with a tyrosine molecule is attached on the porphyrin ring via an urea link, because the affinity of dioxygen might be stronger compared to an amide link [21]. Moreover, in complexes 2 and 3 an axial pyridine ligand is covalently attached to the porphyrin in order to block the lower face of the porphyrin ring. Additionally, it has been found that models bearing covalently attached Fe axial ligands, appear to enhance further their stability [16]. Thus, two different substituted pyridine molecules were used in order to investigate which one will better coordinate to the iron. Preliminary structural investigation was attempted by comparing the ¹H NMR chemical shifts of the free ligands and final molecules. The spectra comparison showed that the tridentate ligand is not suspended over the porphyrin plane and that the 3-pyridine base in model **3** is oriented closer under the porphyrin ring compared to 2-pyridine of model 2 [20].

3.2. Iron metalation reactions

Subsequently, metalation reactions of compounds 1-3 were preformed, using iron dibromide (FeBr₂), under anaerobic conditions, following the same experimental conditions for all models [22,23]. In a glove box iron dibromide was added to a solution of porphyrin in dry THF and the mixture was stirred at 60 °C for 12 h. Insertion of iron induce a color change of the solution from purple to orange, characteristic for ferrous porphyrins. After completion of the reaction, we exposed the solution to the atmosphere and the Fe was immediately oxidized from the +2 to the more stable +3 oxidation state. During this oxidation iron can be complexed with atmospheric oxygen and various oxygenated species can be formed. The separation of the above species is difficult, thus extractions with dilute solution of HCl were carried out [24]. With this procedure a chlorine atom was coordinated with iron and the dimer units were splitted. Consequently, we obtained exclusively monomeric species, where iron was pentacoordinated and had an axial chlorine ligand. The structures of iron porphyrins **1Fe-3Fe** are shown in Scheme 1. For the metalation reaction we used a 10-fold excess of iron dibromide, therefore Fe could also coordinate to tridentate ligand (Cu coordination site). However, this coordination is weak and the iron is liberated from that site, due to protonation of nitrogen atoms by the use of HCl extractions [25]. The incorporation of iron is confirmed by UV-Vis spectroscopy [26] and TLC chromatography. The Soret band of the porphyrin ring was shifted to shorter wavelengths, characteristic of iron binding with the four pyrrolic nitrogens. Also, fewer Q bands and a decrease of extinction coefficient (ε) were observed. Moreover, ¹H NMR spectra of compounds 1Fe-3Fe were obtained. The electronic configuration of Fe^{III} is [Ar]3d⁵ and possesses unpaired electrons in both high-spin and low-spin complexes. As a result, the proton signals in the ¹H NMR spectrum were spread from 80 ppm down to 0 ppm, giving broad peaks that were difficult to assign. High-resolution mass spectrometry (HRMS) and elemental analysis supported the identification of the derivatives.

3.3. Copper metalation reactions

In the next stage, metalation reactions with Cu were performed. The insertion of Cu is done at the iron porphyrins, due to harsh conditions used for Fe metalation. Otherwise copper metal would be replaced by iron. For the introduction of copper, Cu^{+2} was used [24]. To a solution of porphyrin derivatives in MeCN/ethanol, equimolar quantity of $[Cu(CO_2CH_3)_2]$ in MeCN was added. The mixture was stirred at 60 °C for 10 min and the product was obtained pure after precipitation using pentane. The metalated Cu–Fe products (Scheme 2) could not be submitted to further purification due to weak coordination of Cu ion. The compounds could not be fully characterized by NMR spectroscopy, because Fe that is present is paramagnetic. HRMS aided in the identification of the formed complexes and the results were in agreement with the target compounds.

3.4. ¹H NMR studies

As referred above, even though NMR spectroscopy can not be used to fully characterize the metalated complexes, it is a useful technique to determine both the oxidation state and the spin of iron [27]. All NMR spectra of models **1Fe–3Fe** that were obtained showed that the oxidation state of iron is +3 and is high-spin pentacoordinated, since in all cases a signal due to pyrrolic protons is present at around 80 ppm. Following, we investigated the possible coordination of the proximal base as a fifth ligand for the iron atoms of porphyrins **2Fe** and **3Fe**, when Fe is at +2 oxidation state. It is well known in the literature that iron porphyrins in which iron



Scheme 1. Free-based and metalated models 1-3.



Scheme 2. Metalated complexes 1FeCu-3FeCu (the Cu counter anions are not represented).

is hexacoordinated appear to have a diamagnetic NMR spectrum and the pyrrolic protons appear at about 9 ppm [28]. Initially the oxidized porphyrin **3Fe** was diluted in air-free CDCl₃ and NMR spectra were obtained (Fig. 2). A peak at 79.31 ppm is present, due to pyrrolic protons, which is characteristic of high-spin Fe⁺³ porphyrins. Subsequently, excess of carbon monoxide and a solution of Na₂S₂O₄ in air-free D₂O were introduced in the NMR tube. After stirring the solution the organic phase changed color from brown to red due to the reduction of iron in the +2 oxidation state. Then the NMR spectrum obtained corresponds to a diamagnetic complex, since there are no peaks above 10 ppm. The chemical shift of the pyrrolic protons has shifted at 8.61 ppm. The complex that is formed is low-spin, with total spin *S* = 0 and the Fe is six coordinated [29]. The above results show that in complex **3Fe** pyridine is coordinated to Fe⁺² and CO is the sixth ligand that induces an extra stability to the complex. Additionally, the possibility that the sixth axial ligand is another CO molecule is not likely [30].

The same experimental procedure was followed in the absence of CO. The NMR spectrum obtained was exactly the same with the initial spectrum of the oxidized **3Fe** showing a peak at 79.31 ppm, therefore this complex is reoxidized to iron +3 quite fast. In general, pentacoordinated complexes of reduced iron porphyrins are oxygen sensitive and there are only a few reports in the literature that such compounds have been isolated and characterized at room temperature.

Moreover, the above experimental procedure was followed for complex **2Fe**. The initial NMR spectrum of the compound showed pyrrolic proton signals at 7502 ppm, characteristic of oxidized



Fig. 2. (a) ¹H NMR spectrum of oxidized complex 3Fe, (b) ¹H NMR spectrum of reduced model 3Fe in the presence of CO. Solvent used CDCl₃, at 299.2 K.

high-spin iron porphyrins. After the addition of CO and reduction of the porphyrin it was not observed any difference at the NMR spectrum, indicating that pyridine remains unbound. Consequently, the above findings showed that the pyridine axial ligand coordinates with iron in the case of **3Fe** porphyrin, but not in the case of **2Fe**. These results are in accordance with the previous structural investigation that was done using ¹H NMR spectroscopy for compounds **2** and **3** [23].

3.5. UV-Vis absorption studies

UV-Vis spectroscopy was used in order to study iron metalated models. Our first goal was to investigate the possible, axial pyridine ligand binding to iron atom. Therefore, we reduce the iron porphyrins and then compare the UV-Vis spectra of both reduced and oxidized forms of the models. The reduction of iron was achieved with Na₂S₂O₄ in anaerobic conditions using deoxygenated solvents. Porphyrin 3Fe was first dissolved in deoxygenated chloroform and the UV-Vis spectrum was obtained, showing a Soret band at 415 nm and a Q band at 506 nm. Then, in the cuvette saturated aqueous solution of Na₂S₂O₄ was added and the mixture was stirred. UV-Vis spectrum was obtained, and both the Soret and O bands were shifted to 434 nm and 533 nm respectively (Fig. 3). This red shift was due to the reduction of iron and the coordination of the axial pyridine ligand with the iron. Continuing, the above procedure was repeated using model 2Fe and the UV-Vis spectrum after the addition of the reducing agent showed no shift of the Soret band. This indicates that in the case of 2Fe the axial pyridine does not coordinate with Fe. Moreover, in order to examine if one of the pyridines present at the copper binding site coordinates with Fe, we followed the above procedure using 1Fe. The results showed that the Soret band did not shift, therefore there is no coordination of pyridine to Fe.

Our next objective was to study the oxygen binding to iron models. Compound **3Fe** was used to study O_2 binding, since is the only model of the three that has an axial iron ligand. This is in accordance with the catalytic active site of the enzyme of cytochrome *c* oxidase. Following, the reduced complex **3Fe** was exposed to the atmosphere and successive UV–Vis spectra were obtained. A shift to smaller wavelengths is observed, due to oxygen binding. Iron binds molecular oxygen from the atmosphere and at

the same time is oxidized. This procedure lasts about 7.0 min and the Soret band of the product appears at 421 nm and the Q band at 510 nm. It is known that pentacoordinated iron porphyrins that contain bulky groups and a base as an axial ligand, form an intermediate Fe^{+2} – $[O_2]$ upon reaction with oxygen [31]. Oxygen binding is reversible since bubbling argon into the solution gave back the reduced complex. The derivative remains stable for about 1 h, where after that time the Soret band is shifted to smaller wavelengths. Finally at the end of 2 h the Soret band appeared at 418 nm and the Q band at 508 nm, similar absorption bands with the initial **3Fe** (Section 2). In conclusion, model **3Fe** is capable of binding oxygen and forms a quite stable derivative. Therefore, it can be used to further studies in order to understand the mechanism of oxygen binding.

3.6. Electrochemical studies using rotating ring-disk electrode

Following, these porphyrin derivatives were assessed as promising enzyme mimics electrochemically using a rotating ring-disk electrode (RRDE) in the presence of molecular oxygen. Our aim was to compare the catalytic activity of the newly synthesized compounds. The goal of this investigation was firstly to understand the role of copper in the catalytic activity, and secondly to observe if the presence of an axial ligand provides an extra stability to the complex. On the one hand, the main advantage of this technique is its ability to detect H_2O_2 by a ring current at the anode (made of a platinum ring). It is important to note that the H₂O₂ detected at the anode was produced by the reduction of dioxygen at the cathode (composed of a graphite disk) and had migrated significantly. On the other hand, a disadvantage of this technique consists in the irreversible adsorption of the catalyst (the synthetic model) on the surface of the electrode. Indeed, there is no direct way to observe how the catalyst evolves during the catalytic process. In order to carry out the electrochemical experiments, the molecules were adsorbed on the disk electrode, which was a section of a high ordered graphite rod, with the planes of graphite being perpendicular to the surface of the electrode (edge-plane graphite electrode, EPGE). The potential of this modified graphite electrode was scanned from high to lower values so as to record the voltammogram for the reduction of O₂. The graphite electrode was surrounded by a platinum ring electrode, the potential of which



Fig. 3. Successive spectra of 3Fe during its reduction. At the oxidized form Soret band 415 nm and at reduced form 434 nm.

was set at fixed value (1.0 V) such that H_2O_2 eventually produced at the disk was oxidized to O_2 . Catalytic studies of compound 1 are shown in Fig. 4. First, compound 1FeCu was studied (Fig. 4, curve a), and from comparison with the 2e⁻ reduction wave on a bare EPGE, the number of electrons exchanged per O₂ molecule was 3. H_2O_2 is detected through its oxidation, as soon as O_2 is reduced from the current increase at the platinum electrode encompassing the graphite disk. This means that the 2e⁻ and 4e⁻ reduction mechanisms occur simultaneously. From the study of complex 1Fe without Cu (Fig. 4, curve b), a reduced catalytic activity was observed since there was a decrease of electrons in the reduction reaction from 3 to 2.4. Moreover, in the case of 1Fe more H₂O₂ was detected. Therefore, the presence of Cu ion favors the 4e⁻ reduction of oxygen to water. Possibly in model 1FeCu copper is present in a right electronic environment that allows the interaction with oxygen bound on Fe porphyrin. Due to this interaction O-O bond is splitted easier favoring 4e⁻ mechanism and the amount of H₂O₂ is less. These results are in accordance with the



Fig. 4. RRDE voltammograms (a) 1FeCu, (b) 1Fe, and (c) reduction of O_2 from bare graphite electrode.

studies of Karlin et al. in which a similar model to **1Fe** was studied in the presence or absence of a copper ion. In the case of the two models under similar experimental conditions, they observed a $2e^-$ and $4e^-$ reduction of O_2 which is in accordance with our findings [32]. They also observed that the complex that contained copper appeared to have a slightly increased catalytic activity and at the same time there was a decrease at the H_2O_2 that was released. Models **1Fe** and **1FeCu** were unstable and decomposed after successive scans. After carrying out a second scan with the same electrode reduction of the catalytic activity was observed, that approached the one of the bare graphite electrode. The decomposition of the catalyst resulted the poisoning of the platinum electrode from the catalyst molecules. As a result the current intensity can be used in order to arise only qualitative results.

The most precise method of determining the number of electrons that participate in the reduction reaction of oxygen is the study of the current intensity of graphite electrode. For that reason at the voltammograms of models 2 and 3 (Fig. 5), is presented only the current of the electrode where the molecule is absorbed. Compounds 2 and 3 show similar catalytic activity (Fig. 5). In the case of molecules that have metal ions (Fig. 5, curves a and d), oxygen reduction takes place at negative potential values (-0.1 V), where the number of electrons required for the reduction of oxygen is \sim 2.5. The presence of copper did not affect the catalytic activity of these models (Fig. 5, curves b and e), therefore Cu is not involved in the catalytic pathway. Boitrel and co-workers also suggested that the presence of copper in the distal coordination site is not essential for the 4e⁻ reduction of O₂ to H₂O [33]. Moreover, Collman and co-workers observed that copper does not affect the kinetics of the catalysis or the stability of the catalysts [34].

Catalysts **2FeCu** and **3FeCu** reduce oxygen via two mechanisms $(2e^- \text{ and } 4e^-)$, however their catalytic activity is lower compared to catalyst **1FeCu**. The reduction take place at lower potential values (lower than the ones that O_2 is reduced from the bare graphite electrode) and the number of electrons that are exchanged during the reaction is decreased. Since models **2** and **3** contain a pyridine group compared to **1**, it seems that this axial ligand is possibly responsible for the decrease of the catalytic activity. Nevertheless, these results should be explained carefully, because different substitution of the models can affect the adsorption of the catalyst on graphite electrode. The reduced adsorption could result the decrease of the catalytic center at the surface of the electrode, fact that would lead in the reduction of the catalyst at the surface



Fig. 5. RRDE voltammograms (a) 2FeCu, (b) 2Fe, (d) 3FeCu, and (e) 3Fe. Curves (c) and (f) reduction of O₂ from bare graphite electrode.



Fig. 6. Voltammograms obtained after successive scans for 2FeCu (left) and 3FeCu (right).

of the electrode can not be determined due to decreased stability of these models. The decomposition of the catalysts is quite fast, as a result the concentration of the active molecules on the electrode surface to vary during a voltammogram. In previous studies it has been reported by Collman et al. that models that the axial ligand of Fe is covalently linked to porphyrin ring appear further stability [35]. Therefore, comparison of experimental data of compounds **1–3**, can give as an insight on the possible effect of the axial ligand at the stability of the catalysts.

After successive scans models 2FeCu and 3FeCu (Fig. 6) lost their catalytic activity. Compound 1FeCu showed similar behavior which decomposes after successive scanning. Subsequently, even though the models have structural differences, they appear to have similar stability, so the axial ligand does not seem to reinforce their stability. The actual mechanism of the decomposition of the catalysts has not been determined. First it was proposed that H₂O₂ was responsible for the decomposition of the catalyst, nowadays it has been suggested that the destruction of the catalyst includes the formation of hydroxyl radical ('OH). These radicals are more reactive as far as thermodynamic and kinetics is concerned compared to H₂O₂, and they are formed during the homolytic cleavage of O-O. Consequently, the decomposition of the synthetic models is attributed to the formation of hydroxyl radicals. The radicals are very reactive thus they do not reach platinum ring, and instead they decompose the catalyst. Therefore, the study of the stability of an electrocatalyst during O₂ reduction can comprise a quantitative measurement of its selectivity towards the heterolytic cleavage in relevance to the homolytic cleavage of O-O bond. Moreover, based on the above findings some conclusions can be made about the catalytic activity of the models bearing Cu ions. The reduced selectivity of the models that do not contain Cu is not because they can not reduce oxygen to water, but due to their fast decomposition. Absence of Cu the homolytic cleavage of O-O is favored resulting a better catalytic activity and selectivity towards 4e⁻ reduction of oxygen to water.

4. Conclusions

In conclusion, metalated porphyrins **1FeCu–3FeCu** were effectively synthesised, containing a covalently attached tridentate ligand site, a tyrosine molecule and a proximal base. UV–Vis and NMR spectroscopies were used in order to investigate the coordination of the axial pyridine ligand with iron. Furthermore, the complexes were studied for their ability to reduce dioxygen to water on graphite electrodes in contact with an aqueous solution. All models reduce oxygen via two mechanisms (2e⁻ and 4e⁻), and have low stability. The lack of stability was more profound in models bearing axial ligands **2FeCu** and **3FeCu**. Finally, the presence of copper in the case of **1FeCu** increases the catalytic activity of this model favouring 4e⁻ mechanism of oxygen to water, compared to **1Fe**.

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