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Research paper

A question of flexibility in cytochrome *c* oxidase models

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ABSTRACT

The structure-property relationships were compared for the iron and iron-copper complexes of two functional cytochrome *c* oxidase models, **1** and **2**, both constructed upon a phenanthroline-strapped porphyrin bearing respectively pyridyl or picolinyl built-in proximal and distal ligands. The behavior of these heme models in the absence and in the presence of copper was studied by ^{1}H NMR, UV–visible absorption, EPR, Raman and FTIR spectroscopies, electrochemistry in solution and deposited on a rotating ring-disk graphite electrode. The distal binding site within the phenanthroline pocket of both **1** and **2** is available for the coordination of exogenic ligands, yet the oxygen binding affinity is higher for all complexes of **2** than for **1**. Despite this difference, [**1Fe^{II}Cu^{I}**] more efficiently reproduced the electrocatalyzed reduction of oxygen to water than [**2Fe^{II}Cu^{I}**]. The oxygenated complexes of both iron(II)-copper(I) species mimic the ability of cytochrome *c* oxidase to reversibly bind O_2, as shown by competitive binding studies in the presence of CO. Differences in the binding and electrocatalytic properties of these models stem from difference in rigidity of scaffolds upon binding of both the proximal and distal ligands, as well as from the bulkiness of the distal ligand.

1. Introduction

Cytochrome *c* oxidase (C*c*O) is the terminal enzyme in the respiration processes of mitochondria and aerobic bacteria and performs the four-electron reduction of oxygen into water. This redox process is fueled by four cytochrome *c* cycles and coupled to ATP synthesis [1]. The active site for oxygen binding comprises a ferrous heme (heme a_3) and a cuprous copper center (Cu_B) separated by ~5 Å in the fully reduced active site of the enzyme [2,3]. Several functional and structural mimics have been studied to establish the respective roles of iron, copper and the influence of amino acids such as tyrosine located in the vicinity of the proximal site, mostly by comparing the functional behavior of the models with that of the enzyme [4–8]. In general, the function of hemoproteins is fine tuned by the distal residue that controls the approach, position and polarization of exogenic axial ligands. Several groups have successfully reproduced such a controlled environment by using porphyrin derivatives that bear caps or straps, which mimic amino acid functions [4,7,9].

In the particular case of functional models of C*c*O, special attention has been devoted to ligands able to bind copper in both +1 and +2 oxidation states. Depending on the history of each research group in this field, imidazoles, *N*-alkylimidazoles or pyridines were utilized as pendant ligands for copper(II/I) and were linked to the porphyrin architecture via various connectors [8,10,11]. As a result of these numerous variations, a rational comparison of each model's behavior is still very difficult. Over the years, our group has used a phenanthroline-strapped porphyrin [12] as a multi-purpose starting material to explore fields ranging from enzyme mimics to self-assembled porphyrin wires [13]. Because of the unique features of the phenanthroline-strapped architecture, it has been possible to compare C*c*O models in which the copper binding site is almost constant [14]. The work described hereafter shows how small architectural variations of the organic scaffolding affect both the exogenic ligand binding and the reactivity of the iron center.

In the presence of molecular dioxygen, the previously reported ditopic ligand **1** [15] (Fig. 1) provided a realistic mimic of the first oxygenated intermediate in the catalytic cycle of C*c*O [16]. However, although the iron-copper complex [**1Fe^{II}Cu^{I}**]$^+$ catalyzed the reduction of oxygen to water by a predominant four-electron mechanism, our attention was caught by the apparent lability of the proximal pyridyl ligand that was observed during titration

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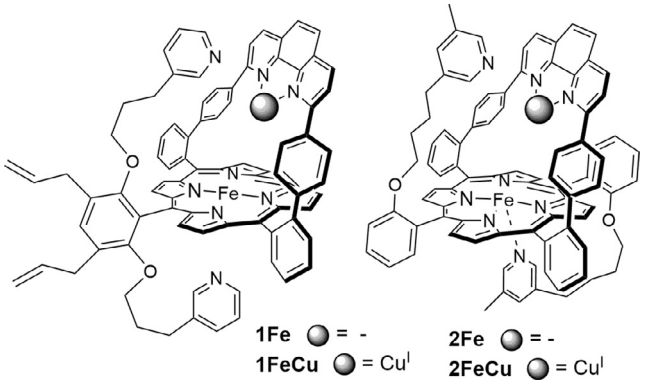

Fig. 1. Cytochrome *c* oxidase models built around a phenanthroline-strapped porphyrin. The phenanthroline pocket provides the distal binding site, whereas the open face of the porphyrin is the proximal site.

experiments with various imidazoles (*vide infra*) and assigned to tension in the pendant ligands. To reduce this constraint, model **2** (Fig. 1) [17] was designed such that the proximal and distal ligands moved independently of one another. In addition, the alkyl chains, from the *meso*-phenyl rings to the picoline ligands, were lengthened by one methylene unit to provide a fully flexible scaffolding. A major drawback of compound **1** was the coordination of the distal pyridine to iron(II) within the phenanthroline pocket, giving a hexacoordinated iron(II) that was relatively inert towards oxygen binding. Replacing the pyridines with a bulkier picoline in **2** would hopefully favor the formation of a pentacoordinated iron (II) complex and in turn vacate the distal site for oxygen binding within the phenanthroline pocket. Hereafter, the coordination geometries, as well as the binding properties at the distal site of iron and iron-copper complexes of models **1** and **2** towards a series of exogenic ligands, including imidazoles, oxygen and carbon monoxide, are compared by NMR, EPR, UV-visible IR spectroscopies and electrochemistry.

2. Results and discussion

2.1. Fe^{III} complexes

The iron(III) complex [**2Fe^{III}**]**Cl** and the previously reported [**1Fe^{III}**]**Cl** [15] were prepared quantitatively, according to a modified literature procedure [18], by treatment of the corresponding free base ligands with $FeCl_2$, followed by oxidation with air. The pentacoordination of both iron(III) complexes, [**1Fe^{III}**]$^+$ and [**2Fe^{III}**]$^+$, was confirmed by ^{1}H NMR, EPR and UV–visible absorption spectroscopies. ^{1}H NMR spectra clearly showed paramagnetic high spin iron(III) complexes with the β-pyrrolic protons near 80 ppm. The paramagnetic, high spin pentacoordinated nature of both complexes was confirmed by characteristic effective *g* values (5.86–5.89) and (2.05–2.06) in the EPR spectra of frozen samples. Finally, the Soret region of electronic spectrum displayed absorptions typical of iron(III) complexes, with an absorption at 424 nm for [**1Fe^{III}**]$^+$ [15] and 428 nm for [**2Fe^{III}**]$^+$.

The availability of the distal coordination site on the central Fe^{III} core was studied by both UV–visible absorption spectroscopy and EPR by adding imidazole derivatives to [**1Fe^{III}**]$^+$ and [**2Fe^{III}**]$^+$. Large changes of the electronic spectra upon the addition of imidazole, 2-methylimidazole or *N*-methylimidazole to solutions of each iron (III) complex in CH_2Cl_2 at 298 K (see Supporting information) suggested the formation of hexacoordinated iron(III) species. Job plots for [**1Fe^{III}**]$^+$ with each imidazole clearly confirmed the hexacoordinated geometry, with two imidazoles per iron porphyrin. For [**2Fe^{III}**]$^+$, absorption spectral changes were minimal after the addition of 1 equivalent of each imidazole derivative. Even in the presence of a large excess of the imidazole guest, there was no evidence for the formation of 1:2 [**2Fe^{III}**]$^+$:imidazole complexes. The coordination of imidazole or 2-methylimidazole within the distal pocket of both [**1Fe^{III}**]$^+$ and [**2Fe^{III}**]$^+$ was further demonstrated by modifications of the phenanthroline's absorption band near 280–290 nm due to the formation of hydrogen bonds between the imidazole NH and the nitrogen atoms of the phenanthroline [16]. No such spectral variation was observed during the titration of [**1Fe^{III}**]$^+$ with *N*-methylimidazole, for which hydrogen bonding within the phenanthroline pocket is unlikely.

The binding constants of [**1Fe^{III}**]$^+$ and [**2Fe^{III}**]$^+$ with imidazole guests (Table 1) were then calculated by statistical processing of the titration data sets. Porphyrin [**2Fe^{III}**]$^+$ forms only 1:1 complexes with all three imidazoles, indicating that one of the two pendant picoline arms remains tightly bound to the iron(III) center and is not exchanged by the competing imidazole substrates. For [**1Fe^{III}**]$^+$, the competing formation of both 1:1 and 1:2 ligand:substrate complexes was systematically observed. The formation of 1:2 complexes is attributed to a relatively labile coordination of the built-in pyridyl ligand due to strain within the pentacoordinated iron scaffold of the 1:1 complex. Only the binding constant of a 1:2 complex of [**1Fe^{III}**]$^+$ with imidazole could be determined, possibly because the first equivalent of imidazole binds indifferently at the distal or proximal site. At both sites, binding is stabilized by the formation of a hydrogen bond of the imidazole's NH with the nitrogen atoms of either the phenanthroline in the distal pocket or the built-in proximal pyridine.

Evolution of the EPR spectra upon addition of various imidazoles to [**1Fe^{III}**]$^+$ and [**2Fe^{III}**]$^+$ are provided as Supporting information. Addition of one equivalent of nitrogen base led to the formation of the hexacoordinated ferric species with the superimposed high-spin pentacoordinated species for [**1Fe^{III}**]$^+$ (see Supporting information). The "large-g_{max}" feature [19] observed at $g = 3.21$ and $g = 2.24$ and the peak at $g = 4.29$ suggest a mixture of $S = 3/2$ and 5/2 spin states, respectively. As the number of equivalents of ligand increased, the intensity of the fourth peak at $g = 5.89$ decreased; the decrease was more pronounced for the imidazole and 2-methylimidazole ligands. The high spin signal disappeared completely after the addition of only three equivalents of imidazole and 2-methylimidazole. In contrast, addition of 10 equivalents of *N*-methylimidazole merely reduced the peak's intensity by roughly half (see Supporting information). This difference suggests a better stabilization of the imidazole-iron complex by hydrogen bonding between the NH proton of imidazole or 2-methylimidazole and the two nitrogen atoms of the phenanthroline binding site. In contrast, the binding of *N*-methylimidazole is less favorable due to the absence of an NH proton. This result satisfactorily follows the same trend as the association constants obtained from UV–visible absorption titrations.

In contrast, for [**2Fe^{III}**]$^+$ the intensity of the EPR signal assigned to high spin pentacoordinated species decreased significantly upon addition of only one equivalent of the various imidazoles (see Supporting information). This result highlights the role of the picoline's methyl substituent that prevents binding of the distal picoline to the iron(III), thus facilitating the coordination of the nitrogen ligand. The more basic character of the proximal picoline ligand also precludes its displacement by the competing imidazole, which is consistent with the UV–visible titration observations. Overall, the EPR and UV–visible absorption binding studies with both iron(III) complexes were particularly useful to confirm the availability of the distal binding site for small exogenic ligands. Oxygen binding was subsequently investigated with the iron(II) derivatives.