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Preliminary Communication

A model for the 'shuttling ligand' of cytochrome *c* oxidase. The synthesis and spectroscopic characterization of allyl–Fe(TPP) and Cp–Fe(TPP) ☆

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Abstract

Cytochrome *c* oxidase catalyzes the reduction of molecular oxygen to water and has been the subject of many studies. The focus of previous studies has been the characterization of intermediates that occur in the reduction of dioxygen to water. Cytochrome a_3, one of the hemes in cytochrome *c* oxidase, contains a farnesyl tail which is thought to 'shuttle' in and out of the binuclear site during the course of the catalytic reaction. While in the binuclear site, the tail is thought to be coordinated to the heme iron center in a π-allyl fashion. With this thought in mind, the synthesis and characterization of two model complexes for this π-allyl intermediate are reported here.

Keywords: Cytochrome *c* oxidase; Ligand shuttle; Allyl complexes; Iron complexes; Porphyrin complexes

1. Introduction

Cytochrome *c* oxidase, the terminal enzyme complex of the mitochondrial respiratory chain, catalyzes the four-electron reduction of molecular oxygen to water. The enzyme isolated from bovine heart contains two hemes (cytochrome a and a_3) and two associated copper atoms (Cu_A and Cu_B). The cytochrome a_3 and Cu_B are located in close proximity and form the site where molecular oxygen is bound. The complex intramolecular electron transfers that occur between cytochrome a/Cu_A and the binuclear center cytochrome a_3/Cu_B, where the formal reduction of molecular oxygen to water occurs, have been studied by various spectroscopic techniques [1]. The focus of this work has been on characterizing the intermediates that occur in dioxygen reduction, which is essential for elucidating the chemical mechanism of the redox processes catalyzed by the enzyme. Cytochrome a_3 contains a farnesyl tail (Scheme 1(a)) which has been suggested to interact with the iron center in one of the catalytic steps. This interaction would probably be of the π-allyl–iron type (Scheme 1(b)) and with this in mind we have synthesized two complexes which might resemble this intermediate [2].

2. Experimental

In the preparation, manipulations were carried out under an argon atmosphere by using standard vacuum-line and Schlenk techniques or an inert atmosphere glovebox. The solvents were freshly distilled under nitrogen from the appropriate drying agents. Fe(TPP)Cl (TPP = tetraphenylporphyrin) was purchased from Strem Chemicals and used as received. AllylMgBr (allylmagnesium bromide) and NaCp (sodium cyclopentadienylide) were purchased from Aldrich and also used as received.

Allyl–Fe(TPP) (allyl = CH_2CHCH_2) was prepared as follows. Fe(TPP)Cl (250 mg, 0.355 mmol) was dissolved in 25 ml of benzene and 0.90 ml of 1 M allylMgBr was added dropwise. After addition of the allylMgBr, the solution slowly changed to deep red in color and some white precipitate formed. The mixture was stirred for 12 h to insure completion and then the solution was filtered through celite. The solvent was then removed in vacuo leaving a deep red solid. This solid was then redissolved in n-hexanes and hexamethyldi-

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☆ This paper is dedicated to Professor F.A. Cotton on the occasion of his 65th birthday.

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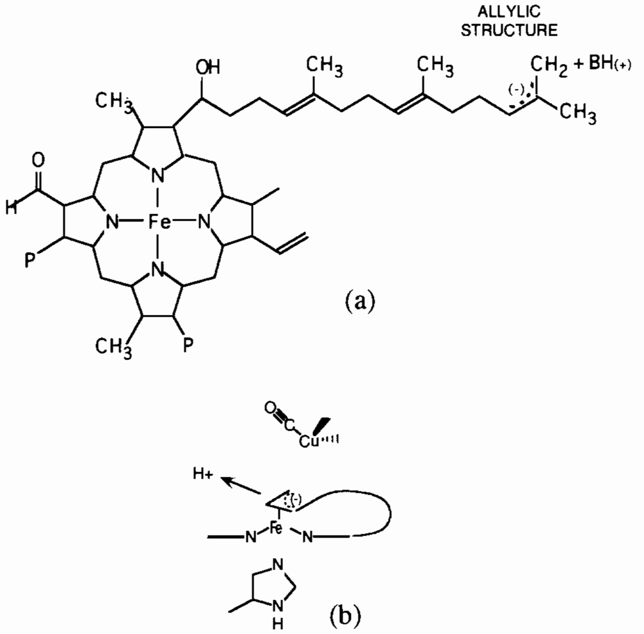

Scheme 1.

siloxane was added to precipitate 150 mg (68%) of allyl–Fe(TPP). *Anal.* Calc.: C, 79.5; H, 4.7. Found: C, 79.1; H, 4.6%. UV–Vis (nm): 405sh, 428vs, 458s, 565m, 604w, 627w, 821br. Emission (nm): 610m, 630s, 670sh.

Cp–Fe(TPP) (Cp = cyclopentadienyl) was prepared as follows. Fe(TPP)Cl (250 mg, 0.355 mmol) was dissolved in 25 ml of benzene and 78 mg of NaCp (dissolved in 5 ml of benzene) was added dropwise. After addition of the NaCp the solution slowly changed to deep red in color and some white precipitate formed. The mixture was stirred for 12 h to insure completion and then the solution was filtered through celite. The volume was then reduced in vacuo and n-hexanes added to precipitate 150 mg (58%) Cp–Fe(TPP). *Anal.* Calc.: C, 80.2; H, 4.5. Found: C, 79.8; H, 4.6%. UV–Vis (nm): 408vs, 430sh, 570m, 614w. Emission (nm): 610sh, 635m, 650s, 670sh.

Excitation for the resonance Raman experiments was provided by the 457.9 nm line of an Ar^+ ion laser (Spectra Physics Model 2025). To minimize photodegradation, samples were spun at a rate of 50 rpm and laser power was kept below 100 mW. Scattered light was focused into a Jobin Yvon Model S3000 triple monochromator (slits set to 3–4 cm^{-1} resolution). The samples were prepared in an inert atmosphere glovebox and flame-sealed in 5 mm NMR tubes upon removal.

3. Results and discussion

In spite of the large numbers of iron porphyrins, porphyrins containing Fe–C bonds are relatively rare and were previously confined to alkyl-type complexes [3–5]. We embarked on the effort to synthesize complexes in order to show that this type of complex could exist and was stable. The synthetic procedures employed to obtain these complexes are simple and the properties they exhibit leave little doubt about their identity in the absence of crystallographic structural data.

The allyl–Fe(TTP) and Cp–Fe(TPP) complexes have been characterized by UV–Vis, emission and resonance Raman spectroscopies. These spectroscopies exhibit many features which are typical of iron porphyrin complexes and a few which are unique to this new class of complexes.

The UV–Vis spectra of both these complexes exhibit the usual metalloporphyrin bands (400–450 nm) and several new bands (450–900 nm) which are not typical. The absorption spectrum of allyl–Fe(TPP) is shown in Fig. 1. These transitions may be due to new ligand-to-metal charge-transfer bands or some form of π–π interaction between the allyl fragment and the porphyrin. Further study of these transitions and other spectroscopic studies may be useful in the unambiguous assignment of these spectra.

Both of these complexes emit when excited with light below 500 nm. This is a very unusual property for an iron porphyrin and the electronic interactions which give rise to this behavior are not understood. The excitation dependence suggests that the emission comes primarily from the new absorbance at around 450 nm.

Resonance Raman spectra have been obtained for the allyl–Fe(TPP) complex and 457.9 nm excitation was found to give the greatest resonance enhancement. The resonance Raman spectrum of allyl–Fe(TPP) is shown in Fig. 2. The spectrum shows many of the modes which are typical for iron porphyrins and give some structural information [4,6]. The two most important modes, from which one may draw some information, are at 1365 and 1565 cm^{-1}. The positions of these two bands indicate that the iron is substantially out of the porphyrin plane and that the oxidation state is III. However, one must keep in mind that these are only empirical correlations and only structural and magnetic data will give absolute parameters. There are also two modes that are unusual for iron porphyrins. These modes are located at around

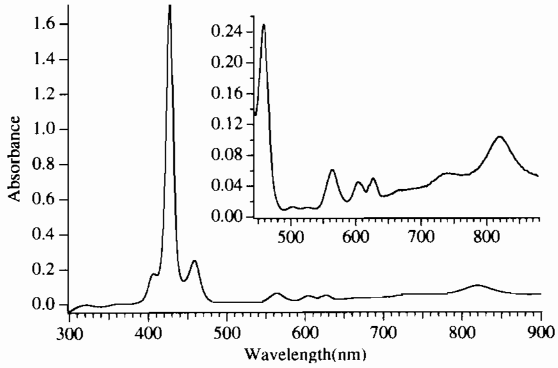

Fig. 1. UV–Vis spectrum of allyl–Fe(TPP).

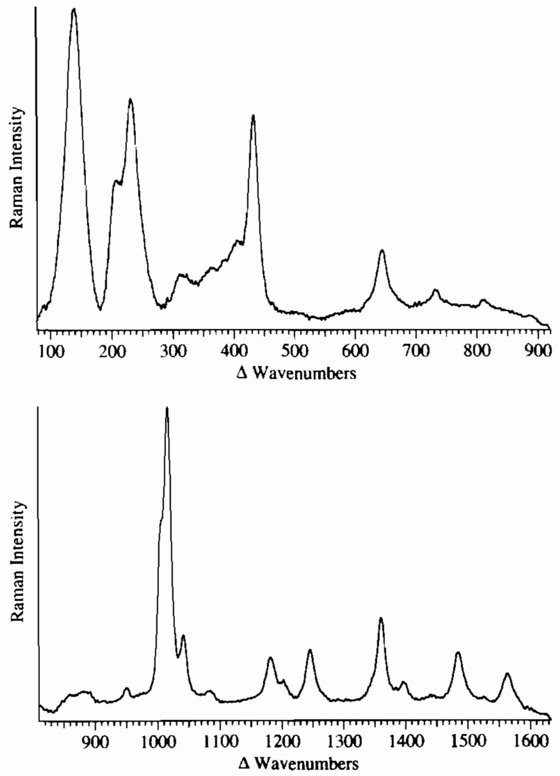

Fig. 2. Resonance Raman spectrum of allyl–Fe(TPP).

400 and 1200 cm^{-1}. Based on other metal–allyl complexes, these have been assigned tentatively to the Fe–C and C–C–C stretching, respectively [7].

The spectroscopic evidence presented here supports our assignment of these new complexes as allyl–iron porphyrin species. Work is currently underway to crystallize the complexes reported here or synthesize other derivatives which may be easier to isolate in crystalline form. Efforts are also underway to secure labeled allylMgBr species in order to assign the Raman spectra more accurately.

Acknowledgements

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