A Ruthenium Octaethylporphyrin Analogue of the Cytochrome P-450 Active Site

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Cytochrome P-450, found in both liver microsomal and bacterial systems, has been extensively studied over the past two decades [1]. These enzymes function as monoxygenases in both systems. Much of the interest in Cyt. P-450 stems from its possible involvement in mammalian carcinogenesis [2]. The most widely accepted mechanism for oxygenation by Cyt. P-450 involves the intermediacy of a ferrous–superoxide or ferric–peroxide species *1* [3]. Models for this proposed intermediate have been prepared by the reduction of a ferric

$$\begin{matrix} Fe^{+2} & \longleftrightarrow & Fe^{+3} \\ | & & | \\ O_2^- & & O_2^= \end{matrix}$$

1

octaethylporphyrin-superoxide complex [4] and also by the addition of superoxide to a ferrous tetraphenylporphyrin complex [5]. However, neither complex was reported to oxidize hydrocarbons. This report details our efforts to prepare the ruthenium porphyrin analogue of *1*.

Our first approach involved the electrochemical reduction of a preformed ruthenium(II) octaethylporphyrin dioxygen adduct *2* [6], A 10^{-6} *M* solution

$$\begin{matrix} Ru^{II}OEP & \longleftrightarrow & Ru^{III}OEP \\ | & & | \\ O_2 & & O_2^- \end{matrix}$$

2

of *2* in dimethylformamide (DMF) containing 0.1 *M* tetramethylamino hexafluorophosphate as supporting electrolyte was reduced using a Princeton Applied Research Model 174 electrochemical system utilizing a three electrode geometry: a working platinum electrode, a counter platinum electrode, and a saturated calomel electrode (S.C.E.) as reference. Complex *2* was generated at 0 °C by bubbling oxygen through a DMF solution of ruthenium(II) octoethylporphyrin, Ru(II)OEP, [6] followed by purging with argon to remove excess oxygen. The reduction of *2* occurred reversibly at −1.29 volts *vs.* S.C.E.

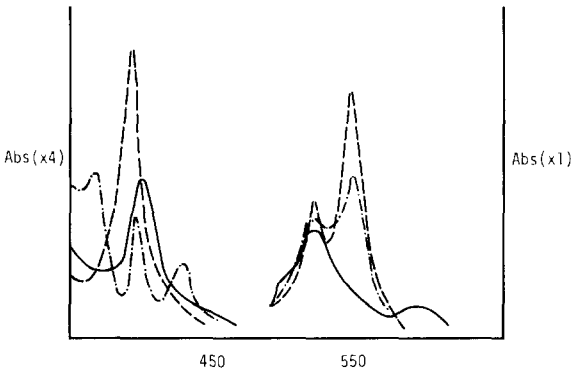

Fig. 1. Visible absorption spectra: *3*, $Ru^{II}OEP(RSH, CO)$, (– –); *4*, $Ru^{II}OEP(RSH)_2$, (——); *5*, $Ru^{II}OEP(RS^-, CO)$, (– · –). All spectra were recorded as *ca.* 10^{-5} *M* dimethylformamide solutions.

We interpret this as a one electron reduction of the porphyrin ring such as was observed for ruthenium(II) tetraphenylporphyrin (CO) [7]. Thus it was not surprising that bulk electrolysis of *2* in the presence of a wide variety of hydrocarbon failed to yield any hydrocarbon oxidation products.

Our second approach was to add electrochemically generated superoxide ion to Ru(II)OEP. Addition of Ru(II)OEP[CO]EtOH [6] to DMF/dodecanethiol generates Ru(II)OEP[CO]RSH, *3*, which can be photolyzed to $Ru(II)OEP(RSH)_2$, *4*. Deprotonation of *3* with one equivalent of *n*-butyl lithium generates $Ru(II)OEP[CO]RS^-$, *5*, which exhibits a split Soret band or hyperporphyrin spectra very similar to that reported for $Ru(II)TPP[CO]RS^-$

$$\begin{matrix} RSH & & RSH & & RS^- \\ | & & | & & | \\ Ru^{II}OEP & \xleftarrow[RSH/DMF]{h\nu} & Ru^{II}OEP & \xrightarrow{nBuLi} & Ru^{II}OEP \\ | & & | & & | \\ RSH & & CO & & CO \\ 4 & & 3 & & 5 \end{matrix}$$

[8]. The changes in the visible spectra that occur during these reactions are shown in Fig. 1.

Reaction of complex *4* in DMF/RSH solution with a DMF solution of superoxide, generated by electrochemical reduction of oxygen in DMF [9], resulted in the spectral change noted in Fig. 2. We propose that this reaction generates complex *6* which can be formulated in several different ways as shown below:

$$\begin{matrix} RSH & & RS^- & & RS^- \\ | & & | & & | \\ Ru^{II}OEP & \xrightarrow[DMF]{O_2^-} & Ru^{II}OEP & \longleftrightarrow & Ru^{III}OEP \\ | & & | & & | \\ RSH & & O_2 & & O_2^- \\ 5 & & & & 6 \end{matrix}$$

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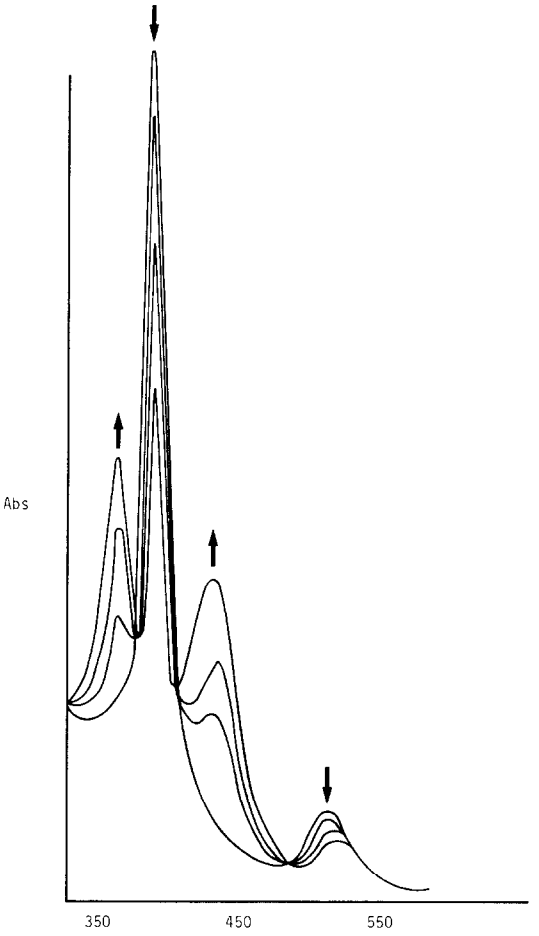

Fig. 2. Visible absorption spectral changes obtained during incremental addition of superoxide (O_2^-)/dimethylformamide solution to ruthenium(II) octaethylporphyrin bis dodecanethiol in dimethylformamide.

Compound *6* would be the ruthenium analogue of the active site in cytochrome P-450. The presence of the split Soret band in *6* indicates that the thiol is probably present as the thiolate. The ruthenium ion lowers the pK_a of the thiol ($pK_a \sim 11$) to a low enough value that the excess superoxide ion (pK_a = 4.8) can function as a base. Addition of a wide variety of bases to *5*, in the absence of superoxide ion, does not generate a split Soret band thus ruling out a $Ru^{II}OEP(RS^-)_2$ formulation for *6*.

Complex *6* is completely unreactive to cyclohexene at temperatures from 0 to 40 °C. The complex is relatively stable at 0 °C but is rapidly destroyed at temperatures above room temperature. The substitution of ruthenium for iron in a model of the cytochrome P-450 active site seems to have effectively stabilized the complex but does not provide any oxidative activity toward cyclohexene.

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