

## Enzyme Mimic Displaying Oscillatory Behavior. Oscillating Reduction of Manganese(III) Porphyrin in a Membrane-Bound Cytochrome P-450 Model System

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Oscillating chemical reactions, in which concentrations of species show a regular periodic increase and decrease, have been the focus of much scientific interest during the last decades.<sup>1</sup> In Nature, oscillations are also known to occur, e.g., in enzymatic reactions like glycolysis,<sup>2</sup> the peroxidase-oxidase reaction,<sup>3</sup> and reactions of immobilized acetylcholine esterase and papain.<sup>4</sup> While the glycolytic reaction is characterized by a complex degree of coupled enzymatic cycles, the aerobic oxidation of NADH catalyzed by the enzyme peroxidase-oxidase is the best understood, homogeneous oscillatory single enzyme system known so far. Oscillatory behavior in synthetic enzyme models has not been previously reported. Such models might provide insight into the factors that are responsible for the oscillations in natural systems.

We report here that a membrane-bound cytochrome P-450 enzyme mimic (Figure 1) exhibits oscillating behavior in the reduction of manganese(III) porphyrin. Previously we showed that the rhodium complex **2** is an efficient catalyst for the reduction of manganese porphyrin **1** by sodium formate.<sup>5</sup> This system is capable of epoxidizing alkenes in the presence of molecular oxygen with turnover numbers that lie in the same range as those observed in Nature.<sup>5</sup>

Manganese porphyrin **1** and rhodium complex **2** were incorporated into zwitterionic L- $\alpha$ -dipalmitoylphosphatidylcholine (DPPC) in the same way as reported previously.<sup>5</sup> Gel permeation chromatography showed that the porphyrin and the rhodium complex were bound to the bilayers of the vesicles. Furthermore, it was shown that the structure of the vesicles as

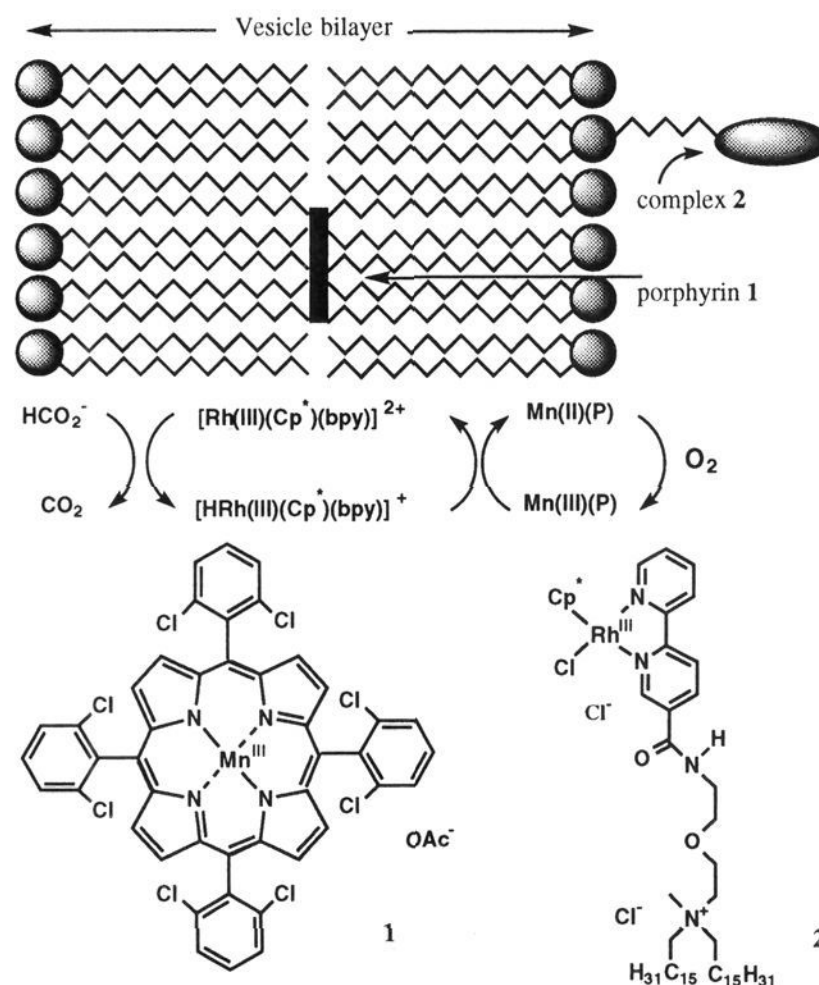


Figure 1. Schematic representation of the cytochrome P-450 mimic and the redox couples and components.

observed by electron microscopy and the phase transition temperature of the bilayers (38 °C, differential scanning calorimetry) was not affected by the incorporation of **1** and **2**. Fluorescence spectroscopic investigations as reported previously<sup>7</sup> revealed that the porphyrin moieties were not aggregated at the porphyrin to lipid ratios used in the present study.

In an initial series of experiments, we investigated the influence of the concentration of formate ions and the rhodium complex, as well as the effect of the temperature, upon the reduction of the porphyrin.<sup>6</sup> The reduction of manganese(III) porphyrin at different formate concentrations follows a Michaelis–Menten type rate law as we showed elsewhere.<sup>7</sup> At formate concentrations exceeding 0.08 M the rate of reduction reaches a constant value. The Michaelis–Menten behavior suggests that the coordination of formate to the rhodium center takes place in an equilibrium.<sup>8</sup> The rate-determining step is the formation of a rhodium(III) hydride species which, once formed, reacts rapidly with the manganese(III) porphyrin. Arrhenius plots of the reduction of **1** at high formate concentrations showed no inflection at the phase transition temperature of the bilayers. The activation energy was calculated from the slope of the plots and found to be  $87 \pm 5$  kJ/mol. The reduction rate of **1** varied linearly with the rhodium concentration; e.g., for [Rh]:[Mn] = 1:1,  $k_0 = 12 \pm 2$  nmol·L<sup>-1</sup>·s<sup>-1</sup>; and for [Rh]:[Mn] = 10:1,  $k_0 = 120 \pm 10$  nmol·L<sup>-1</sup>·s<sup>-1</sup>.

So far all reduction experiments have been carried out under an argon atmosphere. In air at 48 °C and at a formate concentration of 0.25 M the reduction of manganese(III) porphyrin could not be observed at [Rh]:[Mn] < 10:1. Under aerobic conditions the reoxidation of the manganese(II) porphyrin is considerably faster than its reduction. At ratios [Rh]:[Mn] > 10:1, however, net reduction of **1** took place. Oscillations in the concentration of manganese(II) porphyrin were detected at [Rh]:[Mn] = 10:1, as illustrated in Figure 2. After

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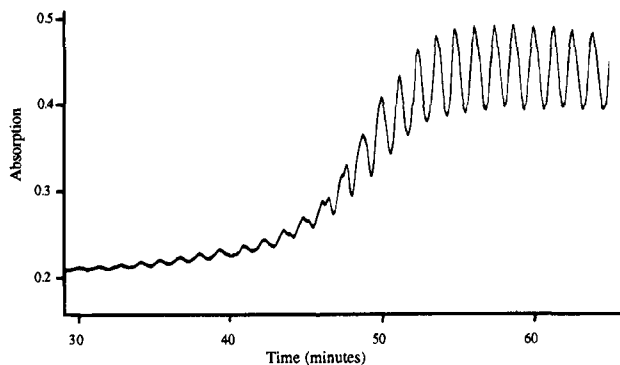
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(6) Reduction experiments: The desired amounts of stock solutions of **1**, **2**, and DPPC in chloroform were mixed in a test tube. The solvent was evaporated under a stream of nitrogen to leave a homogeneous film. This film was solubilized in 100  $\mu$ L of ethanol/THF (1:1, v/v) and injected into 2.5 mL of ethylmorpholine/sodium formate buffered water that had been purged with argon for 30 min at 48 °C. Conditions, experiments with formate ions: 2.4  $\mu$ M **1**, 12  $\mu$ M **2**, 910  $\mu$ M DPPC in ethylmorpholine (50 mM)/sodium formate (varied between 0.016 and 0.150 M) buffer (pH = 7.0),  $T = 48$  °C. Temperature experiments: 2.4  $\mu$ M **1**, 7.2  $\mu$ M **2**, 910  $\mu$ M DPPC in ethylmorpholine (50 mM)/sodium formate (150 mM) buffer (pH = 7.0);  $T$  was varied between 299 and 336 K. Experiments with rhodium complex: 2.4  $\mu$ M **1**,  $2.4 \times n$   $\mu$ M **2** ( $n = \text{Rh}:\text{Mn}$  molar ratio), 910  $\mu$ M DPPC in ethylmorpholine (50 mM)/sodium formate (150 mM) buffer (pH = 7.0),  $T = 48$  °C. Oscillation experiments: 2.4  $\mu$ M **1**, 24.0  $\mu$ M **2**, 910  $\mu$ M DPPC in ethylmorpholine (50 mM)/sodium formate (150 mM) buffer (pH = 7.0),  $T = 48$  °C.

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**Figure 2.** Change in absorbance at 435 nm versus time under the conditions at which the oscillating reduction of manganese(III) porphyrin takes place.

an induction time of ca. 30 min, the manganese(II) porphyrin (UV-vis  $\lambda_{\text{max}}$  435 nm) was gradually formed; and ca. 50 min after the start of the experiment, the onset of oscillations with an oscillatory period of ca. 90 s was observed. When the Mn(III) species was monitored, a complementary retrogression curve was obtained, which indicates that the manganese porphyrin shuttles between the +2 and the +3 oxidation state. A semi-batch reactor with an oxygen-selective Clark electrode was used to measure the oxygen concentration in solution.<sup>9</sup> At the start of the experiment the concentration of oxygen was 5.2

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mg/L, which dropped to almost 0 when the Mn(II) species was formed. Changes in temperature caused the oscillations to cease, since both the reduction and the oxidation reactions are temperature dependent.

At an O<sub>2</sub>/N<sub>2</sub>-gas flow containing 1% oxygen, oscillations were not observed; instead, the manganese(II) porphyrin concentration was found to increase linearly with time. When this concentration had reached its maximum, both the B-band and the Q-band of the porphyrin were found to be shifted bathochromically by 5 nm. This may indicate that after reduction the porphyrin molecules move to a part of the bilayer that has a different polarity. Earlier studies by our group proved that manganese(II) porphyrin is located in the middle of the vesicle bilayer and oriented parallel to the bilayer normal.<sup>7</sup> The charged manganese(III) porphyrin is probably situated at the bilayer/water interface. We tentatively propose that, upon reduction, **1** moves more to the middle of the bilayer. These alterations may lead to small changes in the bilayer packing, which in turn will change the local oxygen concentration. This mechanism may account for the onset of the oscillations.

In summary, we have presented the first example of an oscillation reaction in an enzyme model system. Calculations and further experiments are in progress in order to elucidate the underlying factors causing the oscillatory behavior.

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