

**PHOTOINDUCED REDOX PROCESSES
IN SOME IRON PORPHYRINS
STUDIED BY RESONANCE RAMAN SPECTROSCOPY**

SYNOPSIS

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S Y N O P S I S

Porphyrins and metalloporphyrins play important roles as prosthetic group in a wide variety of biological systems. The photosensitizer and redox chromophore in photosynthetic systems is a magnesium porphyrin. In hemoproteins the iron protoporphyrin IX functional group carries out diverse functions, i.e., as oxygen carrier and storage in hemoglobin and myoglobin,¹ as electron carrier in cytochromes² and as catalytic centre in peroxidases and catalases.³ These manifold functions are controlled by the microenvironment of the heme group provided by the protein pocket. Changes in oxidation, ligation and spin states of the central iron atom with concomitant changes in the stereochemistry of the chromophore are responsible for the diverse physicochemical and biochemical functions of hemoproteins. The cooperative binding⁴ of dioxygen to hemoglobin exemplifies the mode of action of these biomolecules. In an effort to understand the structure-function relationship of these complicated biomolecules, extensive studies on naturally occurring iron porphyrins and their model complexes have been carried out by a wide variety of techniques⁵ at interdisciplinary level.

As the reduced state of iron is often an essential requirement in the functioning of most of the hemoproteins, a variety of techniques have been used to achieve this state in model and natural iron porphyrin systems including chemical,⁶ electrochemical⁷ and photochemical methods.⁸ For a proper understanding of the functioning of hemoproteins and other porphyrin systems, it is necessary to elucidate the basic mechanism of redox processes under different environmental conditions.

The nature of chemical bonds and the geometrical structure of the molecules lend themselves to be effectively probed by vibrational spectroscopy. Selective enhancement of certain vibrational modes of the porphyrin chromophore by Resonance Raman (RR) technique provides unique and important information about the electronic excited states, chemical bonds, ligation states, core size, effects of environment and conformation of the molecules. Extensive investigations on well characterized porphyrins and metalloporphyrins have resulted in important correlations⁹ between Raman frequencies and various stereochemical parameters such as oxidation (of the porphyrin ring or the central metal), ligation and spin states of the central metal atom, core size of the porphyrin ring etc.,.

This thesis describes systematic RR and optical absorption studies carried out on model iron porphyrins in aqueous and organic solvents, in the presence of biologically relevant nitrogenous imidazole bases as axial ligands, in an effort to understand the mechanism of photoinduced redox reactions in these systems. The RR studies on the μ -oxo dimer of simple iron tetraphenylporphyrin, $(\text{FeTPP})_2\text{O}$, formed on dissolution of FeTPPCl in alkaline detergent micelle have enabled us to characterize, for the first time, the oxoferryl porphyrin complex, $\text{TPPFe}^{\text{IV}}=\text{O}$, generated via photodisproportionation of the μ -oxo dimer at ambient and at low temperatures. Our studies on iron tetraphenylporphyrin chloride (FeTPPCl) have shown that under certain conditions reduction at the metal centre takes place on photoexcitation in the presence of specific ligands without the mediation of primary alcohols. Our detailed RR and absorption spectral studies have revealed the presence of an absorption band on the higher energy side of the Soret band in model iron porphyrin complexes under various ligation conditions which arises from a

photoreactive state responsible for photoreduction. This is a significant result for understanding the mechanistic details of photoreduction process in these systems. The studies on photoreduction of iron protoporphyrin (FePPCl, hemin) in ionic and non-ionic micellar media have shown interesting results. While in non-ionic and cationic micelles, photoreduction of hemin in anaerobic conditions in the presence and absence of added nitrogenous ligands was a facile process, in anionic micelle, the same phenomena was observed only in the presence of trace amounts of primary alcohol along with nitrogenous ligands. Individual chapters in this thesis provide details regarding the different aspects of these studies.

This thesis consists of seven chapters:

A general review of Resonance Raman and other related studies of porphyrins and metalloporphyrins form the main content of Chapter 1. Important studies on the photoreactivity of iron porphyrins have been discussed. Some of the pertinent photoredox reactions of iron porphyrins as well as related techniques for creating active intermediates capable of catalytic reactions and *in situ* monitoring of these processes by RR technique have been described.

Relevant theoretical aspects for an understanding of the electronic absorption and Resonance Raman spectra of iron porphyrins are dealt with in Chapter 2.

Details of various experimental techniques used in this study along with a brief description of the methods of sample preparation, the Laser Raman Spectrometer, relevant details of other instrumentation and

accessories are described in Chapter 3. A brief description of the micellar systems used in our work and preparation of samples in them are also given in this chapter.

In Chapter 4 we report¹⁰ our studies on the photochemical behaviour of μ -oxo dimer, $(\text{FeTPP})_2\text{O}$, obtained on dissolution of iron tetraphenylporphyrin chloride, FeTPPCl , in aqueous alkaline detergent micelle, Triton X-100 (TX-100), by RR and UV-VIS absorption studies under different experimental conditions. The 441.6 nm laser excitation of $(\text{FeTPP})_2\text{O}$ in aerobic conditions in the absence of added bases at room temperature yielded the RR spectra typical of the μ -oxo dimer. The same sample in anaerobic conditions at room temperature showed Raman bands characteristic of two species: that of a five coordinated, high spin (5cHS) ferrous species as well as that of a low spin oxoferryl porphyrin complex. The latter was identified by the appearance of its characteristic RR marker bands at $1570\text{ cm}^{-1}(\nu_2)$, $1370\text{ cm}^{-1}(\nu_4)$ and a band at 843 cm^{-1} assignable to the $\nu(\text{Fe}^{\text{IV}}=\text{O})$ axial stretching mode. These modes showed the expected enhancement at low temperature consistent with the higher thermal stability of oxoferryl porphyrin, $\text{TPPFe}^{\text{IV}}=\text{O}$, complex at low temperatures. The polarized nature of the 843 cm^{-1} mode ($\rho = 0.4$) lends further support for its assignment in accord with earlier observations. The enhancement in the intensity of these modes at lower laser powers along with the expected frequency shift on coordination of dimethylformamide trans to the ferryl oxygen have allowed us to assign the 843 cm^{-1} band unambiguously to the $\nu(\text{Fe}^{\text{IV}}=\text{O})$ stretching mode of oxoferryl porphyrin photoproduct, $\text{TPPFe}^{\text{IV}}=\text{O}$. In the absence of added imidazole bases, the 5cHS ferrous complex is inferred to be axially coordinated by a water molecule. To our knowledge, this is the first observation of an oxoferryl species of simple iron

porphyrin having been stabilized at room temperature in aqueous detergent micelle. The 441.6 nm laser excitation of $(\text{FeTPP})_2\text{O}$ in anaerobic conditions in the presence of hindered nitrogenous imidazole bases like 2-methylimidazole (2-MeIm) and 1,2-dimethylimidazole (1,2-MeIm) at room temperature yielded RR spectra typical of a 5cHS ferrous species, or of a six coordinated, low spin (6cLS) ferrous species in the presence of N-methylimidazole (N-MeIm), suggesting that these complexes originate from the same initial ligand-free, four coordinated, intermediate spin ferrous species formed on photoexcitation of the ferric μ -oxo dimer.

In Chapter 5 we present¹¹ our detailed RR studies of photoreduction of FeTPPCl in CH_2Cl_2 , DMSO and in neat 1,2-MeIm in the presence of 2-MeIm and 1,2-MeIm in the first two solvents. When the relative concentration X of 1,2-MeIm ($X = [1,2\text{-MeIm}]/[\text{FeTPPCl}]$) in a 1 mM solution of FeTPPCl in CH_2Cl_2 was low ($X \approx 500$), no photoreduction of the iron porphyrin was observed in anaerobic conditions unless trace amounts ($\leq 1\%$) of methanol was simultaneously present. However, in the presence of low concentrations of 2-MeIm ($X \approx 100$), photoreduction of FeTPPCl in CH_2Cl_2 was observed on irradiation at either 441.6 or 406.7 nm under anaerobic conditions. On the other hand, at higher concentrations of 1,2-MeIm in CH_2Cl_2 ($X > 500$) or in neat 1,2-MeIm, clean photoreduction was observed in aerobic conditions on excitation at 441.6 nm even in the absence of methanol. In DMSO, however, low concentrations of 1,2-MeIm ($X \approx 100$) sufficed to effect photoreduction of FeTPPCl even in the absence of methanol. A systematic study of the absorption spectra of FeTPPCl in the above solvent systems under experimental conditions similar to those used for RR work revealed the presence of a new absorption band in the 315-340 nm region in those complexes for which photoreduction was observed in our

RR study. The appearance of this new absorption band indicates coordination of hindered imidazole(s) or of methoxy group to iron depending on the experimental conditions. We infer that this absorption band, whose most likely origin is a charge transfer (CT) transition from the axial ligand to iron, arises from a photoreactive state responsible for photoreduction. Almost complete photoreduction of FeTPPCl in CH_2Cl_2 in the presence of 2-MeIm observed with 406.7 nm excitation compared to that with 441.6 nm excitation due to higher absorption of the complex at the lower excitation wavelength lends further support for our interpretation.

In Chapter 6 we present¹² detailed RR and optical absorption studies of iron protoporphyrin IX chloride (hemin) solubilized in aqueous non-ionic Triton X-100 (TX-100), cationic cetyltrimethylammonium bromide (CTAB) and anionic sodium dodecyl sulphate (SDS) detergent micelles, where we have employed RR technique for the first time to monitor the photoreduction of hemin under excitation at 441.6 and 457.9 nm. We have observed that in TX-100 and CTAB in anaerobic alkaline conditions hemin is photoreduced under 441.6 and 457.9 nm excitations leading to the formation and stabilization of four-coordinated, intermediate spin (4cIS), ferrous species along with the presence of 5cHS, ferric and ferrous species in minor quantities. While absorption spectra did not reveal any evidence of axial ligation of the hindered imidazoles to ferric hemin in any of the micellar systems used at alkaline pH conditions, their coordination to the 4cIS ferrous species formed by laser excitation of hemin in TX-100 and CTAB in anaerobic conditions is indicated by the RR spectra characteristic of the 5cHS or 6cLS ferrous complexes in the case of 2-MeIm and imidazole ligands respectively. However, in SDS photoreduction of hemin was observed only in the simultaneous presence of 2-MeIm and trace amounts of ethanol

($\approx 1\%$). This is interpreted as due to the formation¹³ of an ethoxy moiety in the presence of 2-MeIm and hemin and its coordination at the axial position trans to the hydroxyl moiety in the ferric complex and transfer of charge from the ethoxy group to the iron centre on photoexcitation.

From a study of the photoreduction process as a function of concentration of the detergent and pH, it has emerged that the hydroxyl ion coordinated monomeric hemin encapsulated within the micelle is the photoreducible species and that in TX-100 and CTAB photoexcitation at $\lambda \leq 458$ nm results in electron transfer from the hydroxyl ion to iron yielding the ferrous species. Attempts have been made to rationalize the observed absence of photoreduction of hemin in SDS under otherwise identical experimental conditions as in the other two micelles, from the point of view of possible effects of the lesser hydrophobicity of SDS micelle, the proximity of the negative surface charges to the iron centre, the deprotonated carboxylic groups of iron-protoporphyrin and of the known effects of alcohol in coordinating to the iron centre in hemin in the presence of 2-MeIm. Absence of photoreduction of hemin on excitation at $\lambda > 458$ nm in the micellar systems indicates a possible link of the process to the high energy Soret transition or a nearby charge transfer transition.

Chapter 7 summarizes the work carried out in this thesis for understanding the mechanism involved in the photoinduced redox processes of iron porphyrins under various experimental conditions. Suggestions for possible future studies as extension of our work and confirmation of the proposed mechanisms and other aspects in this study are also discussed.

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