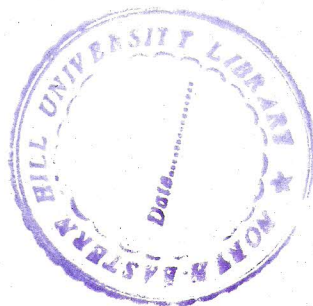


PHOTOPHYSICAL STUDIES ON SOME
PORPHYRINS AND CYTOCHROME-C

A THESIS SUBMITTED
IN
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SYNOPSIS

Porphyrins and their derivatives form an important class of compounds because of their very interesting physico-chemical properties as well as their crucial role in many biological functions. They constitute the chromophore of many biological molecules like hemoglobin, myoglobin, cytochromes, catalases, etc. Apart from their great biological importance, study of physical and chemical properties of these systems is also important in the fields like catalysis,¹ photoconductors, organic semiconductors,^{2,3} superconductors, etc.

Because of their strong absorption in the visible and near UV region, resonance Raman (RR) spectroscopy provides a promising tool for studying molecular and electronic structure of porphyrins and their radicals. Under RR conditions the wavenumbers of the Raman bands exhibit sensitivity to changes in bonding arrangement, central metal, variation in its spin, oxidation and coordination states, etc., while the intensities of the bands are sensitive to the nature of the resonant excited electronic states.

The oxidized or reduced metalloporphyrins (π -cation or anion radicals) serve as transient intermediates in a variety of biological redox processes including light harvesting photosynthetic reaction center in chlorophylls and heme-protein reaction sequences.⁴ The basic process of oxidation and reduction involves electron transfer. Oxidation is the process whereby a substance in a chemical reaction loses one or more electrons. This lost electron by one compound when taken up by another

compound or element leads to the complementary process of oxidation, which is called reduction.

Apart from usual chemical⁵ or electrochemical⁶ methods, porphyrin radicals can also be generated photochemically⁷ by irradiation of the porphyrin solution by selected laser lines in the presence of external electron acceptors or donors. The addition or removal of electron from metalloporphyrins could involve both the porphyrin π -system and the central metal ion. The site of oxidation/reduction is influenced by many factors such as the electronegativity and oxidation state of the central metal,⁸ peripheral substituents at the porphyrin ring,⁹ axial ligands,¹⁰ solvent, temperature and electronic interactions which govern the overlap of electron clouds of the donor and acceptor molecules.¹¹

The current efforts are directed at understanding the electron transfer processes mediated by heme-related proteins in different biological systems. One such heme-protein is cytochrome-c (cyt-c), which shuttles electrons between two membrane-bound proteins, cytochrome-bc₁ complex and cytochrome-c oxidase, of the mitochondrial electron transfer chain. The active site is a mesoheme, which is covalently bound to the protein via two thioether linkages. At pH = 7, the heme iron adopts the Fe^{II}-, or Fe^{III}- low spin state and its two axial positions are occupied by methionyl (Met-80) and histidyl nitrogen (His-18).¹² Since the heme group is buried within the protein, several physicochemical studies have been conducted for delineating various pathways for movement of electron to the heme. Met-80 has been considered to play a crucial role in the redox process,^{13,14} while according to other views, electrons may tunnel to the heme through some aromatic residues,^{15,16} or through the exposed front portion of the heme edge.^{17,18} A mixing of the iron t_{2g}

orbitals with the porphyrin π orbitals may extend the effective metal redox orbital density to the heme edge which may facilitate electron transfer to the metal. However there is no clear mechanism available for electron transfer mediated by cyt-c in spite of so many studies.

Although occurrence of photoreduction and photooxidation has been noticed in some other heme proteins upon laser irradiation at selected wavelengths,¹⁹ nothing is known about their mechanisms, nature of electron donors and acceptors, etc. Ferri-cytochrome-c undergoes five distinct pH – dependent structural changes with pK_a values of 0.42, 2.50, 9.35 and 12.76 producing species with changed axial ligands, unfolding of protein or conversion to dimeric or polymeric forms under different conditions. We, therefore, undertook detailed investigations of the mechanism of photoreduction of cyt-c as a function of pH, oxygen, nitrogen or other inert gases, salt and buffer concentrations, etc. on the yield of photoreduction, using laser excited resonance Raman, UV-visible absorption and fluorescence emission studies. Such studies may help in identifying the possible electron donor to the heme during the electron transfer process, role of excited electronic states, information about transient intermediate species, state of the protein and other factors, which may help in establishing the mechanistic details of photoreduction of cyt-c.

In order to aid and understand the mechanism of photoreduction of cyt-c, we have also investigated the photophysical processes in some porphyrins initiated by selective laser irradiation in the presence of external electron acceptors like quinone, alkylchlorides, tetracyanoethylene or electron donor like substituted imidazoles using

resonance Raman and optical absorption techniques. Attempts have also been made to identify the transient intermediate species.

This thesis describes systematic RR and optical absorption studies carried out on free-base octaethylporphyrin (H₂OEP) and cobalt(II) *meso*-tetraphenylporphyrin (Co^{II}TPP) in the presence of external electron acceptors such as chloranil and quinone, and on cytochrome-c, in an effort to understand the mechanism of photoinduced redox reactions in the systems under selective laser irradiation. With free-base octaethylporphyrin, we carried out systematic studies to explore the detailed mechanism of photo-induced diacid formation in the presence of chloranil and p-benzoquinone as electron acceptors. Our detailed RR studies have revealed that protonation of the neutral free-base to yield the acid derivatives occurs through abstraction of proton from the solvent by an intermediate species formed during the process. When similar experiments were performed on Co^{II}TPP sample, we observed occurrence of photooxidation. The photooxidation of Co^{II}TPP was found to be sensitive to the presence or absence of molecular oxygen in the system. Depending on whether oxygen is present or not, oxidation occurred at the porphyrins ring or at the metal center. The study on cytochrome-c has been initiated with the aim of understanding the specific role of protein environment, conformation and axial ligands on the photoreduction of the heme protein. We monitored the photoreduction as a function of pH, excitation wavelengths, laser power, salt and buffer concentrations.

This thesis consists of seven chapters.

Chapter 1 presents a general review of resonance Raman studies, absorption spectra of porphyrins and other related studies on porphyrins and metalloporphyrins. Some of the important studies on oxidation-reduction products of porphyrins have been discussed in this chapter because of their relevance with this study. Importance of the photon-induced technique employed here for obtaining oxidation/reduction of porphyrin and metalloporphyrin complexes using selective laser irradiation 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 porphyrins and their metal derivatives are given in Chapter 2. Basic ideas about the principle of photophysical process are also given in this chapter.

Chapter 3 presents details of various experimental techniques employed in this study along with a brief description of the sample preparation, the lasers and laser Raman spectrometer, apart from details of other instruments and accessories used in conducting various experiments.

In Chapter 4, detailed studies on photo-induced formation of acid derivatives of free-base octaethylporphyrin under selective laser excitation in the presence of external electron acceptors like p-benzoquinone (p-BQ) and chloranil are presented. When laser line (406 or 441.6 nm) under aerobic or anaerobic conditions excites a solution of H₂OEP in CH₂Cl₂ containing p-BQ or chloranil, certain RR bands of H₂OEP shift to new positions. For example, the wavenumber for the ν_4 and ν_{11} modes shifted from 1369 and 1546 cm⁻¹ to 1388 and 1561 cm⁻¹, respectively. The RR spectral profile of the photo-induced reaction product is similar to one obtained

with chemically prepared H₂OEP diacid. This observation confirms occurrence of photo-induced OEP diacid formation in the system. Other reaction products are generated due to porphyrin degradations irrespective of the presence or absence of molecular oxygen. Diacid formation also occurs in solvents like CHCl₃, C₂H₄Cl₂ and C₂H₂Cl₄, but not in others like CCl₄, CS₂ and C₆H₆. An unexpected observation is that H₂OEP shows no photooxidation in CCl₄ while H₂TPP shows very clean photooxidation, especially considering the similarity between the two compounds. On the other hand, in the simultaneous presence of p-BQ and methanol or ethanol, formation of OEP diacid was observed in CCl₄. In CH₂Cl₂ presence of methanol or ethanol greatly inhibits the formation of diacid. However, with excess concentration of the primary alcohol, we observed formation of OEP monoacid, which shows characteristic wavenumbers at 1385 and 1555 cm⁻¹ for the ν_4 and ν_{11} modes. From the solvent dependent study, we are able to conclude that proton originates from the solvent used and the π -cation radical of OEP generated as transient species is believed to play crucial role in the abstraction of this proton from the solvent, in analogy with the photochemical formation of H₂TPP diacids.^{20,21} The wavenumber shift pattern of the ν_{11} mode (10-16 cm⁻¹), involving mainly C _{β} -C _{β} bond, is in accord with the antibonding character of the a_u (a_{1u} in D_{4h} symmetry) orbital with respect to the C _{β} -C _{β} bond as expected for OEP complexes. However, a large upshift, 15-20 cm⁻¹ in the wavenumber of the ν_4 (pyrrole half-ring stretch)_{sym} mode in which the C _{α} -N and C _{α} -C _{β} bonds stretch out-of-phase, is inconsistent with these expectations since the a_u orbital is nonbonding and bonding, respectively, with respect to the C _{α} -N and C _{α} -C _{β} bonds. From an NMR study,²² it was shown that protonation of

H₂OEP results in deshielding of the N-H protons and hence in a higher electron density on the methine C atom due to migration of negative charge from the anion counter ions to the positively charged porphyrin core. Accordingly, it is suggested that deshielding effect also increases electron densities in the C_α-N and C_α-C_β bonds of the acid derivatives, which get reflected in the large upshift of the ν₄ mode.

In Chapter 5, we report photooxidation of Co^{II}TPP in the presence of p-BQ as electron acceptor probed by resonance Raman and absorption techniques. In CH₂Cl₂ solution, 441.6 nm laser excitation of Co^{II}TPP in the presence of p-BQ under anaerobic conditions results in changes of absorption and RR spectra which are characteristics of Co^{III}TPP⁺. No appreciable formation of the π-cation product was observed under these conditions. However, on allowing molecular oxygen in the system, other spectral changes were observed, which by comparing with chemically prepared Co^{III}(TPP)^{2+•} species, could be identified due to two-electron oxidation product of Co^{II}TPP. Since the formation of Co^{III}(TPP)^{2+•} was observed only in the presence of molecular oxygen, alkylchloride peroxy radicals are suggested to facilitate electron removal from the porphyrin ring. As expected for TPP radical complexes, the ν₂ mode shows downward shift by about 27 cm⁻¹, which is consistent with the a_{2u} character of the radical. Similar π-cation radical formation was also observed in other alkyl chloride solvents such as C₂H₂Cl₄ and C₂H₄Cl₂, but not in CHCl₃, CCl₄ and CS₂. The non-observation of photooxidation in the later group of solvents was attributed to (a) decrease in radical reactivity with increasing number of chloride substituents in the molecules;²³ (b) lower relative dielectric constants which decrease the efficiency of charge separation in the electron transfer process.²⁴

Chapter 6 presents resonance Raman study of photoreduction of ferri-cytochrome-c as a function of excitation wavelengths, laser power, pH, salt and buffer concentrations. During the course of our resonance Raman (RR) studies we had noticed that purified monomeric cyt-c at neutral pH exhibits extensive photoreduction under anaerobic conditions upon laser irradiation in the 400-450 nm region. However, only partial photoreduction occurred at pH 10.1 where the sixth axial methionine ligand is replaced by lysine, and no photoreduction took place after the transitions with $pK_a = 12.76$ and 2.5 where the fifth axial histidine ligand is replaced by other residues. Observation of photoreduction of cyt-c only when it retains its folded conformation suggests that either the histidine ligand or the non-bonding aromatic amino acids, viz., Phe-82 or Tyr-67 could be the possible electron donors in the photoreduction process. Since ionization potential of such aromatic residues is much higher than the energy of the light at 400-440 nm, special mechanism involving a hole-induced electron transfer is proposed. The photoreduction of cyt-c was completely inhibited in the presence of oxygen suggesting active participation of a triplet state in this process. Presence of chloride or phosphate ion also produces inhibiting effect on the photoreduction process, and the effect is related to the type of anion rather than the cation counter part. The dependence of photoreduction on the nature of anion is expected to be a consequence of binding of the ion to some specific cationic amino acids like lysines on the surface and in the proximal part of the protein. Apart from these, detailed experiments were also conducted on the photoreduction as a function of excitation wavelength with constant photon flux. We find that the relative quantum yield for photoreduction of pure cyt-c at neutral pH as a function of excitation wavelengths shows a weak

maximum centered around 410 nm while it increases almost exponentially when excitation wavelength is moved from 380 to 330 nm. This type of behavior is a manifestation of different mechanisms of photoreduction: direct participation of non-bonding aromatic amino acid of the protein in the excitation region below 360 nm and active participation of excited states of the heme group in the 400-440 nm region. During the process of photoreduction, there always exists photo-oxidation, which competes strongly with the photoreduction even under anaerobic condition.

Chapter 7 gives summary and conclusions drawn from our spectroscopic studies in this thesis for understanding the mechanism of photo-induced redox reactions in simple model compounds and in a heme protein. Suggestion for extension of this work for future studies are also made which may help in confirmation of the proposed mechanism and other aspects discussed in this thesis.

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CHAPTER 1