BIOLOGICAL APPLICATIONS OF RAMAN SPECTROSCOPY

VOLUME 3: Resonance Raman Spectra of Heme and Metalloproteins

THOMAS G. SPIRO, EDITOR

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Preface

Some 60 years have passed since the discovery by C. V. Raman (1) of the effect that bears his name. In the decade following this discovery, Raman spectroscopy provided data on the vibrational frequencies of many molecules (2). Only during the 1940s did the introduction of practical infrared (IR) spectrometers lead to the displacement of the more laborious Raman method for routine recording of vibrational spectra. In the prelaser days, heroic measures had to be taken to record the very weak spectra emanating from the Raman effect; this generally involved the placement of a large optically clear sample inside a high-quality discharge lamp (3). The concentration of light power provided by the laser has revolutionized Raman technology (4) and vastly increased its applicability.

Biological molecules have historically been problematic as an arena for vibrational spectroscopy, despite the promise of this method as a probe of molecular structure. Infrared spectroscopy is constrained by the fact that water, the ubiquitous solvent of biology, is a strong absorber of IR radiation. It is a weak Raman scatterer, and, as early as 1938, J. T. Edsall expressed the cautious hope that Raman spectroscopy could be applied to the study of proteins (5). Twenty years elapsed before this hope was rewarded with a "quite faint" photographic Raman spectrum of lysozyme (6). The first laser Raman spectra of proteins were published in 1968 (7). Since then the growth of the literature on the biological applications of Raman spectroscopy has been explosive.

This set of three volumes provides a comprehensive view of a still new and rapidly evolving field through summaries of representative areas by authorities

in biological Raman applications. A major research objective has been to extract information from Raman spectra about the conformations of biological macromolecules: proteins, nucleic acids, and lipids. This is the theme of Volume 1, which considers these macromolecules in isolation and also in organized biological assemblies: ocular lenses, viruses, and membranes. In addition, the collective vibrational modes of macromolecules are considered from a theoretical standpoint, and there is a review of the promising new area of vibrational optical activity.

The introduction of the laser actually had two revolutionary influences on Raman spectroscopy. One was the dramatic relaxation of constraints on the size and optical clarity of the sample and the attendant improvement in signal quality. The other was the ability to produce Raman scattering from absorbing samples, thanks to the minimization of the light path allowed by the directional laser beam. This permitted systematic studies of the resonance Raman effect, which has been an extremely active and productive area from both experimental and theoretical perspectives. The importance of the resonance Raman effect for biological applications is that it can provide dramatically increased sensitivity and selectivity. The theory of the effect is presented in Volume 2, which also explores applications to rhodopsin, to the purine and pyrimidine bases of nucleic acids, to the peptide backbone and chromophoric side chains of proteins, to flavins, and, finally, to the introduction of resonance Raman labels into biological systems. Heme proteins have provided a particularly rich opportunity for resonance Raman studies, and various aspects of work in this area are discussed in Volume 3, which also includes applications to chlorophylls and nonheme metalloproteins.

It is hoped that these volumes give a sense of the high promise that Raman spectroscopy holds for providing unique and important molecular information in biology. They are dedicated to Professor Richard C. Lord, who, perhaps more than any other single individual, has been responsible for the development of this realm of science. His pioneering studies on proteins and nucleic acids have been an inspiration to most current practitioners in the field, and the students that he trained have played an important role in pushing back its frontiers. He is held in the highest esteem by his many friends and admirers, who respond to his wisdom and to his wonderful human qualities. One of his former students, Professor George Thomas, has written the Dedication that appears in Volume 1.

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February 1988

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Resonance Raman Spectroscopy of Metalloporphyrins

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Contents

1 INTRODUCTION

For the past decade and a half, metalloporphyrins have provided Raman spectroscopists with a rich tapestry of resonance phenomena to explore. Several factors conspired to make metalloporphyrins one of the most studied classes of molecules in the modern era of Raman spectroscopy:

1. The extended aromatic system of the porphyrin ring gives rise to two low-lying π-π^* electronic transitions that are conveniently excited with visible lasers.
2. Configuration interaction and vibronic mixing between these transitions produce a variety of interesting resonance effects in the Raman spectrum, which can be examined selectively by tuning the excitation wavelength. These effects have attracted the attention of theorists, and spurred the development of resonance Raman (RR) theory.
3. Metalloporphyrins are available with a wide variety of peripheral substituents, central metal ions, and axial ligands, which introduce systematic changes in the electronic and vibrational spectra and add further richness to the array of resonance phenomena.
4. The vibrational frequencies have been found to be responsive to important aspects of porphyrin geometry and electronic structure.
5. Iron porphyrins are the prosthetic groups of heme proteins, a wide and important class of biological molecules.

A number of other naturally occurring macrocyclic complexes, such as chlorophyll and vitamin B_{12} are closely related to porphyrins. Because of the sensitivity and selectivity conferred by resonance enhancement, these chromophores can be studied in situ, in their macromolecular complexes, their vibrational signatures offering a useful probe of structure.

Biochemical interest has inspired much of the work on metalloporphyrin RR spectroscopy. Indeed, the first spectra (1–3) were obtained for heme proteins. These revealed features of great theoretical interest, including structured excitation profiles and vibrational Raman bands with anomalous polarization (3). The latter phenomenon had been predicted by Placzek (4), some 40 years earlier, in a footnote to his well-known monograph on Raman theory. This prediction was overlooked, however, and the appearance of Raman bands with stronger intensity in perpendicular than parallel polarization, which contravened the polarization rules in all textbooks, came as a genuine surprise to spectroscopists. In addition to these novel characteristics, which reflect the nature of the porphyrin excited states, the early heme protein studies revealed ground state vibrational frequency shifts associated with the ligation chemistry of the

heme group. Much subsequent work on metalloporphyrins has been aimed at establishing structure-spectra correlations. Sensitivities of specific vibrational modes to the porphyrin core size, and to ligation, oxidation, and spin-state of the central metal have been established.

Vibrational analyses, utilizing ^{2}H and ^{15}N isotope substitution, have led to a fairly detailed description of the various in-plane vibrational modes of porphyrins with different classes of peripheral substituents: porphine (5), tetraphenylporphine (6, 7), octaethylporphyrin (8, 9), protoporphyrin IX (10), and porphyrin *A* (11). Metal and ligand isotope substitution has been used to identify bands due to the axial ligands and their bonds to the central metal atom (see especially Chapters 2 and 3 of this volume). These bands are especially useful in monitoring the interactions of the heme group with its protein environment. A beginning has been made in analyzing the porphyrin out-of-plane modes (12–14), which are believed to be responsible for a number of low-frequency RR bands seen for heme proteins. These appear to be quite variable among different proteins and may give important information about the effect of the globin pocket on the heme, when they have been properly assigned. In addition, the out-of-plane force field is an important determinant of dynamic processes in heme proteins, which are currently under intensive study.

In this chapter, the basic character of metalloporphyrin vibrational spectra and the resonance enhancement mechanisms to which they are subject are summarized, and structural influences on the vibrational frequencies are discussed. Succeeding chapters take up applications of resonance Raman spectroscopy to structure and function in specific heme proteins. The reader is also referred to other reviews on heme protein RR spectroscopy (15–19).

2 ELECTRONIC STRUCTURE AND RESONANCE RAMAN ENHANCEMENT

2.1 In-Plane Modes

2.1.1 General Considerations. Porphyrins are aromatic macrocycles with four pyrrole rings connected by unsaturated (methine) carbon atoms at the four corners of a square (Fig. 1). In the porphyrin dianion, with or without a metal ion in its central cavity, there is an inner 16-membered conjugation pathway with 18 electrons; the conjugation is extended by the double bonds connecting the outer atoms of the pyrrole ring. This large aromatic system has a relatively small gap between its valence and conduction bands (~ 2 ev), and metalloporphyrins absorb light strongly in the visible and near-ultraviolet region of the spectrum. Figure 2 shows the absorption spectrum of ferrocytochrome c, which illustrates the classical features of metalloporphyrin spectra: a very intense band, called

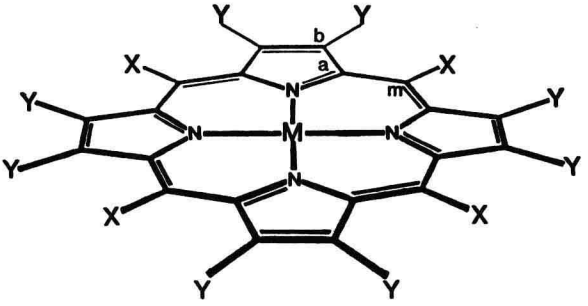

Figure 1. Structural diagram of a D_{4h} metalloporphyrin, with atom labeling. For porphine, X=Y=H, while for TPP, Y=H, X=phenyl. For OEP, X=H, Y=ethyl; this is a model for biological porphyrins, which have X=H and carbon substituents at Y.

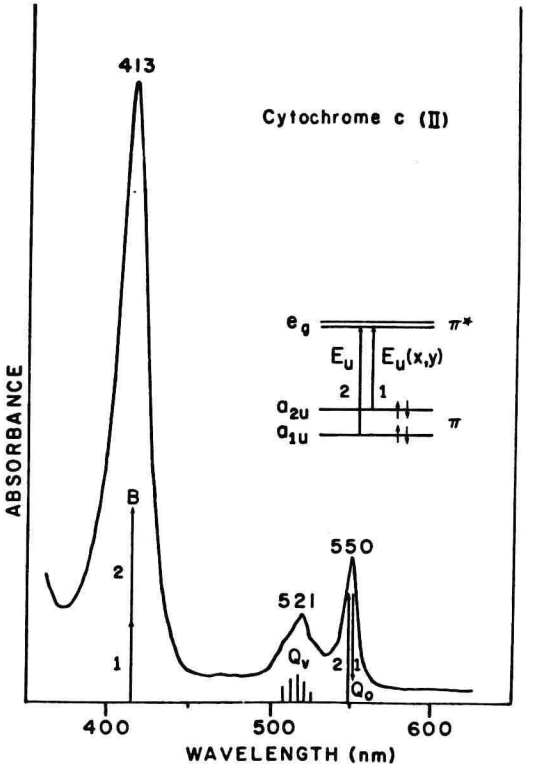

Figure 2. Absorption spectrum of Fe^{II} cytochrome c, with a schematic representation of the four-orbital model for the visible bands. The two π-π orbital excitations, 1 and 2, are subject to strong configuration interaction, with the transition dipoles adding in the higher energy B transition, and nearly cancelling in the lower energy Q_o transition. The Q_v band is the envelope of $0 \rightarrow 1$ vibronic transitions induced by mixing of the Q and B electronic transitions.

the B or Soret band, near 400 nm, and a pair of weaker bands, called Q_O and Q_v or α and β, between 500 and 600 nm. These are best understood with reference to Gouterman's four-orbital model (20) illustrated in Figure 2. In the idealized D_{4h} symmetry of the tetrapyrrole ring, the lowest-lying unoccupied π^* orbitals are degenerate and have e_g symmetry, while the two highest filled π orbitals are of a_{1u} and a_{2u} symmetries. Because the filled orbitals have nearly the same energy, there is a large interaction between the two orbital excitations $a_{1u} \rightarrow e_g$ and $a_{2u} \rightarrow e_g$; the transition dipoles add up for the intense B transition, and nearly cancel for the weaker Q_O transition (whose intensity vanishes completely if a_{1u} and a_{2u} are accidentally degenerate). Some intensity (about 10 percent) is regained for the Q transition via vibronic mixing with the B transition, leading to the Q_v side-band, which is an envelope of vibrational bands built on the Q_O band (some vibrational structure can be discerned, and is better resolved at low temperature). The four orbital model also accounts satisfactorily for the principal features of metalloporphyrin RR spectra, as discussed in the next section.

There are two RR mechanisms of importance, A and B term scattering (21). These are the two leading terms of a Taylor expansion of the Raman polarizability with respect to a vibrational normal coordinate Q. The A term is

$$A = \mu_e^2 \hbar^{-1} \sum_v F_v (\Delta\nu_v + i\Gamma_v)^{-1}; F = \langle j | v \rangle \langle v | i \rangle \qquad (1)$$

where μ_e is the electric dipole moment for the resonant excited state e, of which v is a particular vibrational level; $\Delta\nu_v$ is the difference between the frequency of the level v, of bandwidth Γ_v, and the laser excitation frequency (resonance occurs when $\Delta\nu_v = 0$). F_v is the product of Franck–Condon integrals between the intermediate level v and the initial and final levels i and j, which are usually the 0 and 1 vibrational levels of the ground electronic state. The A term provides the dominant resonance scattering mechanism for allowed electronic transitions, with large values of μ_e^2. The relative enhancements for different vibrational modes depends on the values of the Franck–Condon integrals, which in turn depend on the geometric displacements in the excited state long the vibrational coordinates. Only totally symmetric modes have nonzero Franck–Condon products, and are subject to A term enhancement.

The B term contains the derivative of the transition moment with respect to the normal coordinate, $\partial\mu_e/\partial Q = \mu_e'$

$$B = \mu_e \mu_e' \hbar^{-1} \sum F_v' (\Delta\nu_v + i\Gamma_v)^{-1}; \qquad (2)$$

$$F_v' = \langle j | Q | v \rangle \langle v | i \rangle + \langle j | v \rangle \langle v | Q | i \rangle \qquad (3)$$

and the vibrational overlap function F_v' contains integrals which depend on Q,

as well as Franck–Condon integrals; it can be nonzero for nontotally symmetric vibrations. When the resonant excited state is only weakly allowed, μ_e' can exceed μ_e if there is a nearby strongly allowed transition s, which can lend intensity to the resonant transitions by vibronic mixing. In the Herzberg–Teller formulation

$$\mu_e' = \mu_s \langle s \,|\, \partial H/\partial Q \,|\, e \rangle / \hbar(\nu_s - \nu_e) \tag{4}$$

where $\partial H/\partial Q$ is the derivative of the Hamiltonian with respect to the normal mode, and ν_s and μ_s are the frequency and transition dipole moment of the mixing state. The magnitude of μ_e' diminishes with increasing frequency separation between the two states. The active vibrations are those that are effective in mixing the two states and depend on their geometries. The allowed symmetries are given by the cross product of the electronic transition representations.

A limiting case of vibronic scattering is the Jahn–Teller effect, when the two electronic transitions become degenerate. The mixing vibrations (Jahn–Teller active modes) are strongly enhanced. Jahn–Teller scattering can alternatively be viewed as arising from the A term since $\mu_e = \mu_s$. The foregoing equations based on perturbation theory no longer apply, however, and the vibronic matrix must be diagonalized in a quantitative treatment.

2.1.2 B and Q Band Enhancement. For metalloporphyrins, excitation in the vicinity of the intense B absorption band produces mainly A term scattering (22, 23). Totally symmetric modes of the prophyrin ring (which have depolarization ratios [ρ] less than $\frac{3}{4}$ dominate the spectra. Because the excited state is degenerate (E_u), however, it is also possible to see Jahn–Teller-active modes, of B_{1g} and B_{2g} symmetry ($\rho = \frac{3}{4}$). The intensities of these modes are quite variable. Jahn–Teller activity is closely linked to the configuration interaction between the $a_{1u} \rightarrow e_g$ and $a_{2u} \rightarrow e_g$ orbital excitations. When the a_{1u} and a_{2u} orbitals are exactly degenerate, these excitations contribute equally to the B and Q transitions and Jahn–Teller activity vanishes. This restriction is lifted when the two orbitals have different energies. Cheung et al. (24) have shown that the strength of B_{1g} and B_{2g} mode enhancement in resonance with the B absorption band correlate with the strength of the Q_o absorption band; the latter reflects the diminution in configuration interaction, due to increased $a_{1u} \rightarrow a_{2u}$ energy separation, and consequently the extent of Jahn–Teller activity.

Excitation in the vicinity of the Q bands produces mainly B term scattering, dominated by the vibrations that are effective in mixing the Q and B transitions (3, 25, 26). Since these are both of E_u symmetry, the allowed symmetries of the mixing vibrations are