Resonance Raman Studies of Heme Proteins and Models

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There has been considerable interest in heme proteins for many years. Even so, the electronic and structural changes associated with oxygen binding or electron transfer have not been determined unequivocally [1]. These problems are difficult since biological materials are very complex, thereby necessitating the use of highly sensitive spectroscopic probe which selectively sample the atoms in the vicinity of the active site. Resonance Raman (RR) spectroscopy offers considerable promise in this regard, if the atoms in the site rise to an isolated electronic absorption band [2]. RR spectroscopy has been extensively applied to heme proteins and metalloporphyrin analogues. By using RR, the chief obstacle encountered with biological materials, complexity, is almost totally solved.

Raman spectra are observed when light is scattered inelastically by molecules in solid, gases or liquids. In Raman spectroscopy a molecule is excited by a laser, light is scattered, and the energies of the inelastically scattered photons are analyzed. Raman and RR spectroscopies measure the frequency difference from the exciting line. These frequency differences correspond to vibrational transitions. The Raman and RR range cover the same region as the so-called middle-to-far IR, falling between visible and microwave light [3].

The total intensity of radiation scattered during a Raman process is proportional to the fourth power of the frequency shift and the second of the molecular polarizability or scattering tensor [4]. Raman, i.e., non-resonance Raman, mainly gets its intensity by the frequency shift of the incident radiation. The contribution of polarizability in Raman intensity is very small. However, as the laser exciting frequency in Raman approaches an allowed electronic transition in the molecule being investigated, those normal modes that are vibronically active in the electronic transition exhibit a pronounced enhancement ("resonance") in their Raman intensity.

For example, when a heme protein is irradiated in the region associated with porphyrin localized $\pi \rightarrow \pi^*$ electronic transitions, the porphyrin vibrational mode intensities are enhanced by vibronic coupling. Generally, the Soret band excitation enhances the A_{1g} vibrational modes and α or β excitation enhances non-totally symmetric vibrational modes, i.e., B_{1g} and B_{2g} for porphyrin systems. Asymmetric vibrational modes, A_{2g} in porphyrin systems, show anomalous scattering with a depolarization ratio approaching infinity; these modes are inactive in normal Raman spectra [5].

A number of heme proteins and model systems have been the subject of recent studies and reviews [2,6]. RR was used first by Spiro *et al.* to characterize heme proteins [5]. Correlations were established between the frequencies of some prominent RR bands and the spin and oxidation states of the iron atom in protoporphyrin IX and in mesoporphyrin. Four bands were identified which offer promise as

oxidation and/or spin state makers [7]. Reduction of Fe(III) to Fe(II) leads to a small general lowering of frequencies, while much larger decreases accompanying conversion from low- to high-spin state. The former trend was explained in terms of changes in π back-donation, while the latter trend was attributed to structural changes accompanying the spin state change [8]. By spin and oxidation state markers, oxyhemoglobin was classified as a low-spin Fe(III) heme [7,8]. FeTPP (meso-tetraphenyl porphine) was studied in order to correlate frequency as a function of electronic and structural changes [9,10]. Mansuy et al. found four oxidation and/or spin state marker bands of Fe-TPP complexes and a characteristic band for pentacoordinated derivatives [9]. Since mainly in-plane porphyrin modes are enhanced by RR, all these correlation diagrams used in-plane porphyrin ring modes.

Burke et al. studied the oxyhemoglobin model compound, [Fe(O_2)-(1-MeIm)(TpivPP)], Mono(N-methylimidazole)(dioxygen)meso-tetra-(α,α,α,α-o-pivalamide-phenyl)porphinato iron(II), which has an end-on bend O_2 geometry from x-ray structure [11]. The close similarity of Fe-O_2 in the model compound and in oxyhemoglobin [12] was established by RR and IR. From this similarity they claimed that oxyhemoglobin has the same bent end-on Fe-O-O geometry and appreciable multiple bond character for the Fe-O_2 bond from its relatively high Fe-O_2 stretching frequency [10].

An RR line at 216 cm^{-1} distinguishing the two quarternary structures of Hb, mainly T (low oxidation affinity) and R (high oxidation affinity) state, was found by Kitagawa et al. and was assigned to the Fe-N (HisF8) stretching mode. The Fe-N frequency shift in the R and T transition was interpreted in terms of the tension model of Hb cooperativity [13]. N-Fe stretching in model compound such as Fe(II) picket-fence porphyrin and Fe(II) protoporphyrin IX has also been studied [14].

Recently, influences on the RR frequencies of heme proteins have been studied attributed to more subtle structural changes, such as globin [8] and vinyl-substituent effects [15], axial π-ligand effects [6], and porphyrin core size effects [16].

References

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