UV RAMAN STUDIES OF BIOLOGICAL STRUCTURE AND DYNAMICS AND ENERGY TRANSFER

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The development of UV resonance Raman spectroscopy as a probe of biological structure and function presents opportunities to study both average ground state macromolecular structure as well as molecular dynamics (1). In addition, the Raman excitation profiles and the enhanced vibrational modes detail information on the molecular electronic excited states. These excited states may be biologically relevant for biomolecular function since they may contribute to transition state structure and be involved in electron and energy transfer pathways.

We will review our work in this area and touch on aspects of these issues. For proteins we will examine enhancement of the amide modes and correlate the extent of amide mode enhancement with geometry changes in the excited state (2,3). The strong enhancement of the amide II and amide III modes of polylysine in the random coil, alpha helix and beta sheet conformations demonstrate that the geometry and or the bonding of the amide CO-N linkage is distorted along the C-N bond (Figure 1). The facile photochemical cis-trans photoisomerization and the observed enhancement of the putative amide V overtone vibration indicates that the peptide π^* excited state is twisted compared to the ground state (2). In addition, the excited state appears to be conjugated over the peptide backbond. This result may have great significance for electron transfer rates in peptides as well as for peptide conformation geometry constraints.

We will also review our examination of the conjugation between the peripheral vinyl groups of the heme prosthetic group of hemoglobins and myoglobin. We earlier demonstrated the lack of conjugation of the vinyl groups with the heme pi orbitals; the vinyl stretches are selectively enhanced by the isolated vinyl $\pi \rightarrow \pi^*$ transitions (4). The vinyl group resonance Raman depolarization ratio for excitation in the vinyl group $\pi \rightarrow \pi^*$ is 0.33 for both

heme and a monovinyl heme derivative. This indicates that the vinyl C=C motion is uncorrelated between the two heme vinyl groups. The observed lack of vinyl-heme group conjugation suggests that control of the vinyl group orientation is not likely to be a useful route for the protein to modulate the heme ligand affinities or oxidation potentials.

We have utilized the pulsed laser sources typically used in Raman spectral measurements to examine T_1 relaxation rates in proteins (5,6). The technique, which is called Raman Saturation Spectroscopy, uses the Raman intensities to monitor the concentration of ground state species during the ca 10 nsec laser pulse. Absorption removes ground state population while relaxation repopulates the ground state. The non linear variation of the Raman intensity with increases in the incident intensity, can be used to determine the T_1 relaxation rate (Figures 2 and 3). This T_1 relaxation rate depends upon the radiative and non radiative relaxation rates which are influenced by near neighbor groups which are involved in electron transfer and Forster energy transfer. We will discuss the use of saturation Raman spectroscopy to examine electronic communication between the tyrosines and tryptophans at the hemoglobin subunit interfaces with the heme rings.

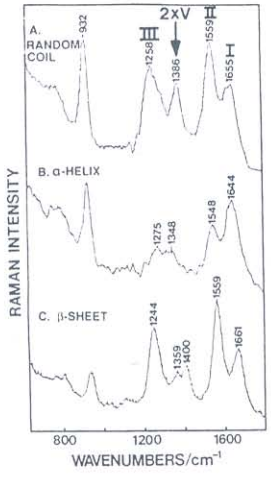

Figure 1. Raman spectra of PLL (0.17 mM) in water at 218-nm excitation, with $NaClO_4$ (0.2 M). The 932-cm^{-1} band derives from ClO_4^- stretching: (A) random coil form, pH = 4.0, 25°C; (B) α-helix form, pH = 11.0, 25 °C; (C) β-sheet form, pH = 11.3, 52 °C.

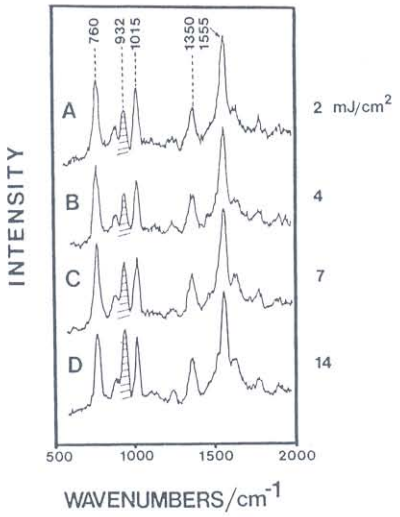

Figure 2. Resonance Raman spectra of TRP (pH 6.5) excited at 225 nm with pulse energy fluxes below 15 mJ/cm^2. Pulse energy fluxes listed in units of mJ/cm^2. Internal standard band shaded.

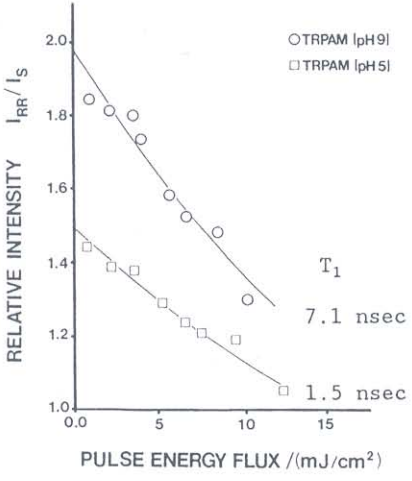

Figure 3. Raman saturation of the 1555-cm^{-1} band of TRPAM (pH 9) (open circles) and TRPAM (pH 5) (open squares) at 225-nm excitation. 0.5 mM analyte, 1 M perchlorate. Solid curves derive from a quantitative model for the saturation. See text for details.

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