UV RESONANCE RAMAN STUDIES OF TEMPERATURE –INDUCED STRUCTURAL CHANGES IN PROTEINS AND POLYPEPTIDES

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Protein amino acid sequences, by themselves, contain all the information necessary to define their unique 3D structure and, thus, their biological function[1]. The protein folding problem refers to how and why a protein adopts a specific "native" conformation. The thorough understanding of the protein folding process is crucial for understanding the functioning of all biological systems.

The UV resonance Raman spectroscopy (UVRRS) gives plenty of *unique* information on intramolecular interactions within a polypeptide/protein molecule. We have recently characterized the Raman spectral ranges which result from an α-helix → random coil transition and we have quantitatively characterized the spectral signatures of the α-helical conformation in model polypeptides and α-helical proteins by UVRRS with excitation of the peptide bond at 206.5 and 204 nm. Recently we examined the UV Raman spectral signatures of a model 21-residue peptide $A_5[AAARA]_3A$ and a protein - hen egg-white lysozyme – and their behaviour (steady-state and kinetic) with temperature changes. Here we report on the steady-state UVRRS study of secondary and tertiary structure changes in other proteins such as horse-heart myoglobin and cytochrome *c* and homopolypeptides with temperature.

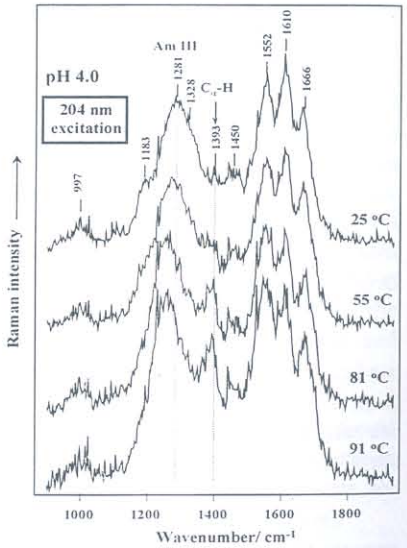

Fig 1. UV resonance Raman spectra of horse heart Myoglobin at various temperatures. Excitation at 204 nm.

Figure 1 shows the changes in spectrum of horse-heart myoglobin with temperature. The process is reversible, *i.e.*, the spectral signature of the native protein recovers upon cooling the heated protein back to room temperature. The

conformational transition detected by changes in myoglobin UV Raman spectrum occurs in the temperature range between ~40 °C to ~60 °C in good agreement with the data obtained earlier by other methods[2,3]. The main changes occur in the amide III and C_α-H stretching vibration bands. The Am III band slightly downshifts and becomes narrower upon heating the protein solution. Simultaneously, the relative intensity of the C_α-H stretching mode increases and also slightly downshifts in the unfolded conformation. Similar changes take place in the UV Raman spectra of other proteins and polypeptides. For example, figure 2 shows the Raman spectra of an Ala-based peptide $A_5[AAARA]_3A$ and poly-L-glutamic acid in α-helical and coil-like conformations. Interestingly, for the Ala-based peptide, the Raman bands are very narrow and their vibronic structure is very well pronounced. This gives us the unique opportunity to characterize interactions within the peptide α-helix during the helix-coil transition in much detail.

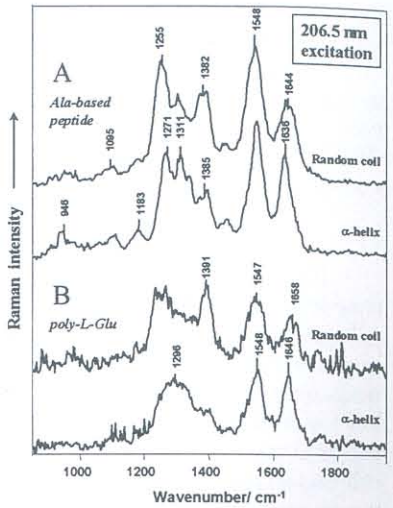

Fig 2. UV resonance Raman spectra of (**A**) Ala-based peptide $A_5[AAARA]_3A$ at 70 °C (94 % random coil) and at -7.4 °C (64 % □-helix), (**B**) poly-L-glutamic acid (M_w=54400) at pH 3.95 at 80 °C (75 % random coil) and at 25 °C (99 % □-helix). Excitation at 206.5 nm.

We applied the quantitative approach developed in our group[4,5] to calculate protein secondary structure and its change with temperature from the UV resonance Raman spectra of the protens.

By varying the excitation wavelength, we selectively enhanced Raman scattering from particular groups (aromatic amino acid residues, and protein prosthetic groups, such as heme) to obtain information about their behaviour in the folding process.

References

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