NANOSECOND TIME-RESOLVED UV RESONANCE RAMAN SPECTROSCOPY OF PROTEIN FOLDING

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Recent time-resolved studies of protein folding using IR, fluorescence and CD spectroscopic techniques established that the first events occur on a nanosecond time scale. We recently demonstrated that steady state UV resonance Raman (UVRR) spectroscopy technique is a powerful tool for characterizing protein secondary structure. We report here the first application of a nanosecond time-resolved UV resonance Raman spectroscopy for kinetic studies of protein folding.

Several approaches are known to initiate protein structural changes. We used the rapid temperature change induced by pump laser pulse to study unfolding. We Raman shifted the YAG fundamental to 1.9 μm (1-st H_2 Stokes shift) to selectively heat the water solvent. Delayed nanosecond UV pulses of 204 nm (5-th H_2 antiStokes shifted YAG 3-rd harmonic) is used for probing.

We report on kinetic study of the temperature-controlled helix-coil transition of alanine-based peptide, $A_5[AAARA]_3A$. The secondary structure of this peptide was characterized by steady state UV Raman spectroscopy in the temperature range from –8 to +80° C.

The alanine peptide was evident from CD studies to have a dominant α-helical secondary structure and shows a non-cooperative transition on a temperature increase. UV Raman spectra of the alanine peptide comprise of five strong bands (Fig. 1, top). Four of them at ca. 1640, 1540, 1380 and 1250 cm^{-1} derive from the amide I, amide II, C_α-H bending and amide III modes, respectively. A narrow band at ca. 1300 cm^{-1} serves as a signature of the peptide α-helical content. The contribution of this band decreases with temperature.

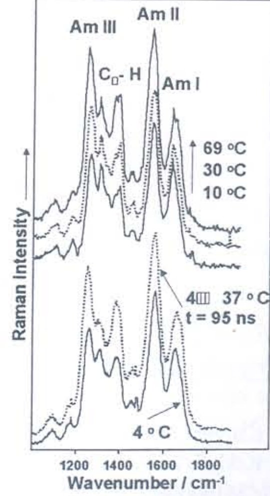

Fig. 1. Steady state (top) and time-resolved (bottom) UVRR spectra of the alanine peptide.

Transient UVRR spectra of the alanine peptide (Fig. 1, bottom) show changes induced by the T-jump which are similar to those observed in the equilibrium spectra measured at different temperatures. The difference spectra shown in Figure 2 are the result of subtraction between transient spectra recorded with and without a pump heating pulse. The difference spectrum for infinite delay time was obtained from the steady state spectra measured at different temperatures. The changes in the Raman spectra at short delay time were mainly due to the peptide temperature increase, without substantial changes in its secondary structure. α-Helix melting at longer delay times results in the appearance of four peaks that dominate the difference spectrum at about 100 ns. The characteristic time, t, for the helix-coil transition was found to depend on the T-jump:

ΔT / ° C	33	43	60
t / ns	180±60	120±40	70±20

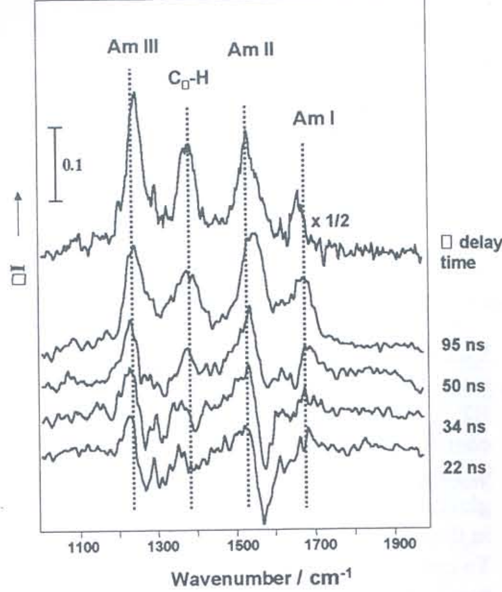

Fig. 2. Transient UVRR spectra of the alanine peptide measured at different delay times after a T-jump of 33° C.

In conclusion, time-resolved UVRR spectroscopy is a powerful tool for kinetic studies of protein folding. Transient Raman spectra provide detailed information about the protein secondary structure and its evolution during the folding/unfolding processes. By varying the excitation wavelength, one can selectively enhance Raman signal from particular groups of protein, for example, from the aromatic amino acid residues or the heme. Isotope substitution may also be used to get a Raman signature from a particular residue. We use a quantitative approach developed recently in our group[1,2] to characterize the protein secondary structure in the transient intermediate states which occur during the folding/unfolding processes.

1. Z Chi, X G Chen, J S W Holtz and S A Asher, Biochem. 37, 2854 (1998)
2. Z Chi and S A Asher, Biochem. 37, 2865 (1998)