LYSOZYME THERMAL DENATURATION: A STEADY STATE AND TIME RESOLVED UV RESONANCE RAMAN SPECTROSCOPIC STUDY

M. C. Sparrow, I. K. Lednev, A. S. Karnoup, and S. A. Asher*
Department of Chemistry, University of Pittsburgh, Pittsburgh PA 15260

One of the major barriers to the understanding of biological function are the pathways through which proteins fold and unfold. There is still little knowledge of how primary sequence encoding specifies folding mechanisms and it is not yet possible to predict protein secondary or tertiary structures solely on the basis of primary structure. Much effort has been put forth toward gaining greater insight into protein folding mechanisms as this will facilitate a greater understanding of biological functions and their role in human health.

Previous kinetic studies of protein folding have mainly been limited by sub-millisecond and longer time scales. However, the first events of protein folding have recently been established to occur on nanosecond time scale[1-4]. A quantitative approach has been developed in our group for characterization of protein secondary structure using UV resonance Raman spectroscopy[5,6]. A nanosecond time-resolved pump-probe apparatus with UVRR detection has also been developed for protein kinetic studies. A T-jump technique utilizing 3ns pulses has been used to initiate protein folding. The T-jump apparatus employs a high pressure H_2 raman shifter to obtain the 1st stokes shift at 1.9μm which provides the heating pulse for the T-jump. A range of excitation wavelengths is provided by a low pressure H_2 raman shifter. In this case, the 5th anti-stokes shift is taken from the YAG 3rd harmonic to provide 204 nm excitation. Variable time delays for the time resolved studies are provided by changing the excitation beam path length.

Here, we report on steady state and time-resolved UV resonance Raman spectroscopic studies of hen egg lysozyme thermal denaturation. Hen egg lysozyme is a relatively small enzyme consisting of a single polypeptide chain of 129 amino acids. The protein amino acid sequence contains several aromatic residues as well as 4 disulfide bridges. A native lysozyme secondary structure is dominated by α-helix and β-sheet motives, which melt in the temperature range of 40-60° C.

By using excitation wavelengths at 206nm and 229nm, we enhanced the resonance Raman signature from both polypeptide backbone and aromatic amino acid residues respectively. The UV resonance Raman spectrum of lysozyme measured with 204-nm excitation is dominated by the amide bands (Fig. 1). A broad peak in the region of the amide III band found for the native protein at low temperature is a signature of predominant α-helical content[5]. Heating the protein results in a cooperative thermal denaturation which gives Raman spectra with two relatively narrow peaks which arise from the amide III and C_α-H bending modes of the random coil structure.

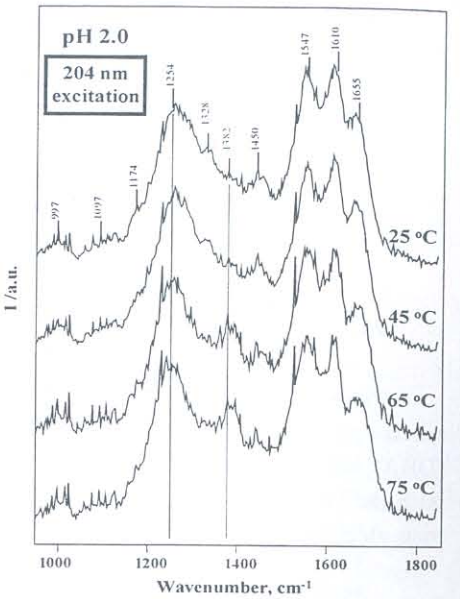

Fig 1. Steady state UVRR spectra of Hen Egg Lysozyme as a function of temperature.

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